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Cover Photograph:

Broad-Beech Fern Phegopteris hexogonoptera, at Wolf's Den in Cherokee County, Alabama

Photo Credit: Jim Loftin, Anniston Museum of Natural History.

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EXCLUSIVE-PCR WITH DENATURING GRADIENT GEL ELECTROPHORESIS: A NEW APPROACH TO IDENTIFY NOVEL ALLELES

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ABSTRACT

AIM: To establish exclusive PCR (E-PCR) with denaturing gradient gel electrophoresis (DGGE) method as a novel approach by identifying *cry4* subclass genes in mosquitocidal strains of *B thuringiensis*. METHODS: *cry4*, a mosquitocidal protoxin gene, was amplified by E-PCR with family and type primers that were designed from known conserved and unique regions, respectively. The E-PCR family products were samples for DGGE. The linear DGGE was performed in 8% polyacrylamide with 20% to 80% denaturant. RESULTS: E-PCR family products amplified from known and unknown subclass *cry4* genes were separated in DGGE, although their DNA sequences differ in only two base pairs (T in position 224 and G in position 394). When using two pairs of family and type primers, the novel subclass genes were identified in tertiary level. A total of three novel subclass *cry4* genes were found from five mosquitocidal *B. thuringiensis* strains. CONCLUSION: E-PCR with DGGE approach is a highly sensitive, specific, and reliable method for identifying potential novel alleles.
KEY WORDS: exclusive PCR; denaturing gradient gel electrophoresis; *cry4* gene; *Bacillus thuringiensis*.

INTRODUCTION

Bacillus thuringiensis is a Gram positive sporulating bacterium that produces proteinaceous protoxins upon sporulation. The protoxins are selectively toxic to invertebrates, mostly insects and nematodes (Beegle and Yamamoto 1997). Genes encoding these protoxins are classified as cry1-28 and cyt1-2, according to differences in sequence and host range specificity (Crickmore et al. 1998). These natural biopesticides typically have a

specific and narrow host range and are limited with respect to the diversity of strains used in commercial products. In addition, with increased use resistant strains of insects are selected. More toxins are needed to target insect pests not controlled by the available biopesticides and to manage the emerging problem of insect resistance (Ferre et al. 1995).

The most important aspect of screening *B. thuringiensis* for novel protoxin genes is to have a method that can rapidly and accurately characterize the novel subclass genes. Protein bioassay analysis is an exhaustive and time-consuming process, because it is necessary to screen all subclass proteins. Immunological analysis with monoclonal antibody does not identify a subclass gene accurately, because the same serotype protein may be from different subclass gene expressions (Beegle and Yamamoto 1992). Southern blot analysis is widely used for searching homologous genes. However, the sensitivity of this method is low for detecting target DNA with low amount or copy number, because signal strength is proportional to the amount of target DNA (Sambrook et al. New York). Analysis by PCR permits rapid determination of the presence or absence of a specific DNA sequence from a target gene. PCR is a good method for the screen because of its sensitivity and reliability.

However, simple PCR is limited to the detection of already-known genes and fails to detect and identify novel genes. To overcome this limitation, exclusive PCR (E-PCR) was applied and has two primer set (Juarez-Perez et al. 1997). One primer designed from DNA sequence from a common region of a class gene is called a family primer that amplifies a family product from all subclass genes. Another primer designed from a DNA sequence selected from a unique region of a class gene is called a type primer that amplifies a specific fragment, a type product, from a single subclass gene. E-PCR identifies an unknown type product amplified from a novel subclass gene by excluded the type products, amplified from all known subclass genes, from a family product amplified from the class gene.

When the length of family product is shorter than that of type products, E-PCR can not be used for the identification. Therefore, we combined with denaturing gradient gel electrophoresis (DGGE) to resolve this problem. During DGGE, PCR family products with the same size but different DNA sequences can be separated (Cariello 1986). Separation in DGGE is based on the decrease in electrophoretic mobility of a partially melted (denatured) DNA molecule in polyacrylamide gels. A melting domain is a stretch of DNA sequence with an identical melting temperature. Once the melting domain with the lowest melting temperature reaches its denaturing condition at a particular position of the denaturing gradient, a transition of helical to partially melted molecules occurs. The migration of this DNA fragment will practically halt. Sequence variation within each domain causes changes of melting conditions and electrophoretic mobilities. Therefore, the same size PCR family products with DNA sequences of novel subclass genes can be identified by their separations at different positions comparing to known subclass genes within a denaturing gradient gel.

To test the efficiency and specificity of E-PCR with DGGE approach, we used a mosquitocidal protoxin class gene, *cry4*, in *Bacillus thuringiensis* as subject. *Cry4* is mosquitocidal specific and with only two subclass, *Cry4A* and *4B*. The goal of this study is to establish the E-PCR with DGGE method as a novel approach to identify mosquitocidal protoxin *cry4* subclass genes in *B. thuringiensis*.

MATERIALS AND METHODS

Experimental bacterial strains. The experimental bacterial strains used in this study are listed in Table 1. These strains, selected after a literature search using Medline, SearchBank, and Cambridge Scientific Abstracts, are a compilation of all mosquitocidal strains of *B. thuringiensis* identified to date. The *B. thuringiensis* strains were kindly donated by Dr. L. K. Nakamura, National Center for Agricultural Utilization Research, United States Department of Agriculture (USDA); Dr. D. R. Zeigler, *Bacillus* Genetic Stock Center (BGSC), Ohio State University; and Dr. M. M. Lecadet, International Entomopathogenic *Bacillus* Center (IEBC), W. H. O. Collaborating Center, Institute Pasteur Paris. *E. coli* DH5α was purchased from Ward's Biological Supply (Rochester, New York).

Long-term stock cultures were stored at -70° C in 35% glycerol and working stocks were maintained on a nutrient poor medium, G-Tris agar, to promote sporulation. Cultures were monitored by phase-contrast microscopy for morphological characteristics, including cell shape, cell arrangement, and spore form. For observing parasporal inclusion bodies, cultures were incubated for 48 hours at 30° C. The inclusion bodies were stained with Coomassie Brilliant Blue (0.25% Coomassie brilliant blue, 50% ethanol, and 7% acetic acid).

B. thuringiensis subsp. *israelensis* was used as a positive control strain, because of its well studied protoxin genes. *E. coli* was used as a negative control strain, because it has no mosquitocidal properties.

Table 1. Bacterial strains

Strain	Serovar	Serotype	Origin	Sources
NRRL HD-5	*kenyae*	H4a,4c	Kenyan	
NRRL HD-11	*aizawai*	H7	Japan	
NRRL HD-30	*canadensis*	H5a	Canada	Schvetzova
NRRL HD-146	*darmstadiensis*	H10		Krieg
NRRL HD-530	*morrisoni*	H8a,8b		de Barjac
NRRL HD-541	*kyushuensis*	H11a, 11c		de Barjac
NRRL HD-542	*thompsoni*	H12		de Barjac
NRRL HD-1014	*neoleonensis*	H24		Padilla
NRRL B-23135	*medellin*	H30	Colombia	Rojas, 1990
NRRL B-23141	*jegathesan*	H28a, 28c	Malaysia	Ho, 1988
NRRL B-23152	*malaysiensis*	H36	Malaysia	Lee, 1991
A4P1	*fukuokaensis*	H3a, 3d, 3e	Japan	Ohba, 1989
T14001	*israelensis*	H14	Israel	
T44001	*higo*	H44	Japan	Ohba, 1989
T29001	*amagiensis*	H29	Japan	Ohba, 1989
DH5α	*E. coli*			

DNA isolation and purification. For DNA isolation, cultures were grown in LB (Luria-Bertani) broth (1% [Wt/Vol] Tryptone, 0.5% Yeast extract, 0.5% NaCl) until mid exponential phase. Cells were harvested by centrifugation (9,000 x g) and washed in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.9). The cell pellet was resuspended in solution with 25 mM Tris, 10 mM EDTA, and 5 mg/ml lysozyme at 60 x concentration. DNA was recovered using an alkaline denaturation procedure as described by Birnboim and Doly (Birnboim and Doly 1979). Cell lysates were treated with RNase A (100 μg/ml) and proteinase K (50 μg/ml) to remove RNA and proteins, respectively. After the isolation, DNA concentration was determined by UV spectrophotometry. The DNA stock solutions (50 - 65 μg/ml) were stored at -20° C in TE buffer. Working solutions were diluted ten times (5 - 6.5 μg/ml) in TE buffer and stored at 4° C.

Design of oligonucleotide primers. All reported subclasses of *cry4* genes are summarized in Table 2 (Crickmore et al. 1998). The DNA sequences as listed in GenBank, the National Center for Biotechnology Information (NCBI), were the basic sequences used for designing family and type primers. The DNA sequences of family forward and reverse primers were selected from two highly conserved regions among all subclass genes following multiple alignment of their DNA sequences. The DNA sequences used for the comparison were retrieved from the GenBank. The comparisons were analyzed using BLAST Sequence Analysis Software that is available at NCBI. The DNA sequences of type forward primers were selected from the variable regions within each subclass gene, following multiple alignment. Family forward and reverse primers were used to yield a family product. A type forward primer paired to a family reverse primer in a PCR yielded a type product. The positions of primers in each subclass gene are listed in Table 2. The DNA sequences of each primer are listed in Table 3.

Table 2. Locations of primer pairs and sizes of PCR products

Positions	Target Genes	GenBank Code	Gene Length (bp)	forward	reverse	Size
Cry4	*cry4Aa1*	Y00423	3543	2932 -	3370	438
	cry4Aa2	D00248	4253	3324 -	3762	438
	cry4Ba1	X07423	3684	2956 -	3394	438
	cry4Ba2	X07082	3684	2950 -	3388	438
	cry4Ba3	M20242	4056	3322 -	3760	438
	cry4Ba4	D00247	4186	3257 -	3695	438
cry4A	*cry4Aa1*	Y00423	3543	1816 -	3370	1554
	cry4Aa2	D00248	4253	2208 -	3762	1554
cry4B	*cry4Ba1*	X07423	3684	2136 -	3394	1258
	cry4Ba2	X07082	3684	2130 -	3388	1258
	cry4Ba3	M20242	4056	2505 -	3760	1255
	cry4Ba4	D00247	4186	2437 -	3695	1258

Primer parameters, namely GC content, length, melting temperature (Tm), self complementarity, and secondary structure, were calculated by Primer! Software from Williamstone Enterprises. The basic sequences were modified as necessary to optimize the parameters (Mitsuhashi et al. 1996). All primers were 20 to 28 bases in length with 32% to 57% (G + C) content (Table 4). The self-complementarity of primers was less than 4 base pairs.

Table 3. DNA sequences of primers

Primers	Sequences
Cry4 family forward	5'-GCA TAT GAT GTA GCG AAA CAA GCC -3'
cry4A type forward	5'- CTT AGT ATC CCA GGG GTA GCA GAA C-3'
cry4B type forward	5'- CGC GAA AGA TGC ATT AAA CAT TGG-3'
Cry4 family reverse	3'- C CTG GAC CTT TAC CCA TAC AGT GCG -5'

Table 4. Physical characteristics of primers

primers	GC content (%)	Tm of GC% (° C)	Standard Tm (°C)	Length (bp)
cry4 family forward	45.83	55.68	63.98	24
cry4A type forward	56.52	58.84	68.26	26
cry4B type forward	41.66	53.97	58.63	24
cry4 family reverse	56.00	60.96	68.48	25

Oligonucleotide primers were synthesized by Life Technologies, Inc. (Rockville, Maryland). Prior to use, the efficiency, specificity, and accuracy of all primers were confirmed by PCR amplification of DNA from *B. thuringiensis* subsp. *israelensis* as a positive control and *E. coli* as a negative control in each PCR reaction.

PCR amplifications. For amplification by PCR, template DNA was used at a final concentration of 0.1 - 0.3 μg/ml. Each oligonucleotide primer was added to a final concentration of 0.2 μM. PCR Supermix from Life Technologies, Inc. included *Taq* DNA polymerase at a concentration of 1U, deoxynucleotide triphosphates (dGTP, dATP, dTTP, and dCTP) at 1 μM, and $MgCl_2$ at a final concentration of 1.5 mM per reaction. The final volume of the PCR reaction mixture was 52 μl.

Amplification was performed in a Perkin Elmer 2400 (GeneAmp 9600) Thermocycler. The initial denaturation was performed at 94° C x 5 minutes, followed by 30 cycles at 94° C x 1 minutes, 52° C x 2 minutes, 72° C x 1.5 minutes, and a final extension at 72° C x 15 minutes completed the PCR.

To enhance the specificity and sensitivity of PCR, the basic PCR program described above was modified to Touchdown or Hot-start programs as necessary. Touchdown is a versatile one-step procedure for optimizing amplification, when primer-template complementarity is not fully known (Hecker and Roux 1996). The program decreases the annealing temperature by 0.5° C every cycle (from 55° C to 45.5° C) to a 'touchdown'

annealing temperature. Then, the amplification continues at this temperature (45° C) for 10 cycles. An optimal annealing temperature for high primer-template complementarity is reached to yield a specific PCR product. Nonspecific annealing and amplification are minimized. Hot-start PCR is used to avoid nonspecific amplification during PCR preparation. In Hot-start PCR, the *Taq* polymerase is inactivated by antibody or added after the initial denaturation step of PCR, which eliminates the nonspecific amplification. In general, the Hot-start program increases the temperature at the initial step of PCR (97° C, 6 min) to initiate the activity of *Taq* polymerase for specific amplification (Chou et al 1992). PCR products were analyzed by 1 - 2% agarose gel electrophoresis.

Simple PCR, amplification with a type forward or a family forward primer paired with a family reverse primer, was used for initial identification of *cry4* genes. Here, E-PCR was used to verify the results of simple PCR. E-PCR products were amplified with a type forward primer paired to a family reverse primer during the first round of PCR; and with family forward and reverse primers during the second round of PCR. Modified E-PCR used type and family forward primers paired with a family reverse primer in a single reaction. Modified E-PCR was used as an alternative method to confirm the results of simple PCR. Repeated PCR was used to eliminate nonspecific amplification. The products of simple PCR (1^{st}) were used as template for repeated PCR (2^{nd}) with the same primers.

DGGE. The linear DGGE gel base was 8% (Wt/vol) polyacrylamide in 0.5 x TAE (0.04 M Tris-acetate, 0.001 M EDTA). The gels were prepared by Biotech Holdings - Gelux. Inc., Hudson, Ohio. The gradients were formed from 20% to 80% denaturant (100% denaturants were 7 M urea with 40% [vol/vol] deionized formamide and 0% denaturant was 6% [Wt/vol] acrylamide stock solutions [acrylamide: N', N', bisacrylamide = 38 : 1]) (Gillan et al. 1998). Initial gels to determine the effective range of denaturant concentration were performed using 20 to 80% denaturant. They were 1.0 mm thick within a cassette of 12 cm (height) x 10 cm (width) and run in a Mini II apparatus (Biotech Holdings - Gelux. Inc.). To predict the same effective range, DNA melting maps of PCR family products were analyzed by a computer algorithm (MELT87) from Massachusetts Institute of Technology. This map predicts a location where DNA fragments will be denatured and stopped to form a band in a denaturing gradient gel (Lerman and Silverstein 1987). The algorithm simulated melting contour probabilities of the PCR products at increasing denaturants. Based on the predicted and experimental tested effective ranges of denaturant concentrations, gels with a narrow denaturing gradient range (from 50% to 60%) were used to identify novel genes.

The sample (60 µl) amplified by a family primer set was denatured by boiling followed by rapid cooling. The gel within a cassette (10 x 10 cm^2) was run in a Single Vertical Mini-Gel Systems apparatus (Cat. #MGV-100, C. B. S. Scientific), 5 hours at 200 volts, in a thermal incubator with a small fan set to maintain an internal temperature of 60° C. After electrophoresis, the gels were incubated for 20 minutes in 0.5 x TAE contained 0.5 mg/L ethidium bromide and photographed under UV light.

RESULTS

Identification of *cry4* genes in mosquitocidal strains of *B. thuringiensis*. DNA

templates from 15 distinct strains of *B. thuringiensis* were used for PCR amplification with *cry4* family, *cry4A* type, or *cry4B* type primers and *cry4* family reverse primer. When amplified with *cry4* family forward and *cry4* family reverse primers, template DNA from *B. thuringiensis* subsp. *israelensis*, subsp. *kyushuensis*, subsp. *medellin*, and subsp. *jegathesan* yielded PCR products of expected size, 438 bp (Figure 1, Lanes 2, 8, 11, 12, respectively). The same primers yielded PCR products of about 700 bp when template DNA from *B. thuringiensis* subsp. *canadensis* was used (Figure 1, Lane 3).

After amplification with *cry4A* type forward and *cry4* family reverse primers, PCR products of 1,554 bp, as expected, were obtained when template DNA from *B. thuringiensis* subsp. *israelensis*, subsp. *canadensis*, and subsp. *kyushuensis* was used in the PCR (Figure 2, Lanes 2, 3, 7, respectively). The PCR products obtained, when template DNA from *B. thuringiensis* subsp. *medellin* was used in the PCR, were about 2 kb size (Figure 2, Lane 10).

When amplified with *cry4B* type forward and *cry4* family reverse primers, template DNA from *B. thuringiensis* subsp. *israelensis* and subsp. *kyushuensis* yielded PCR products of expected size, 1,258 bp (Figure 3, Lanes 2, 17, respectively). The same primers yielded PCR products of about 1,800 bp, when template DNA from *B. thuringiensis* subsp. *canadensis* was used (Figure 3, Lane 3). The results of simple PCR for all target genes are summarized in Table 5.

Table 5. PCR results of *cry4* genes

B. t. **strains**	***cry4* family**	***cry4A***	***cry4B***
Israelensis	+	*Aa*	*Ba*
Canadensis	(+)	+	(+)
kenyae	-	-	-
aizawai	-	-	-
Morrisoni	-	-	-
Kyushuensis	+	+	+
Thompsoni	-	-	-
Neoleonensis	-	-	-
medellin	+	(+)	-
Jegathesan	+	-	-
Fukuokaensis	-	-	-
Malaysiensis	-	-	-
Darmstadiensis	-	-	-
Amagiensis	-	-	-
higo	-	-	-

\+ --- means PCR product with expecting size from unreported distribution
(+) --- means PCR product with unexpected size from unreported distribution
Capital letter --- means PCR product with expecting size from reported distribution

Modified E-PCR confirmed the presence of a *cry4A* gene in *B. thuringiensis* subsp. *medellin*. When using *cry4A* type forward and *cry4* family forward primers paired with *cry4* family reverse primer in a PCR amplification, template DNA from *B. thuringiensis* subsp. *medellin* yielded both family specific and type specific PCR products. The distribution of *cry4A* in *B. thuringiensis* subsp. *israelensis*, subsp. *canadensis*, and subsp *kyushuensis* was confirmed by E-PCR using *cry4A* type and *cry4* family forward primers paired with *cry4* family reverse primers. Both family specific and type specific PCR products were produced.

Modified E-PCR confirmed the presence of a *cry4B* gene in *B. thuringiensis* subsp. *kyushuensis*. When using *cry4B* type and *cry4* family forward primers paired with *cry4* family reverse primer, template DNA from *B. thuringiensis* subsp. *kyushuensis* yielded both family specific and type specific products. Distribution of *cry4B* in *B. thuringiensis* subsp. *israelensis* and subsp. *canadensis* was confirmed by E-PCR using *cry4B* type and *cry4* family forward primers paired with *cry4* family reverse primers. Both family specific and type specific PCR products were produced. A total of three *cry4A* and two *cry4B* unreported distributions were found and verified.

Identification of novel *cry4* genes. PCR *cry4* family products of *B. thuringiensis* subsp. *israelensis*, subsp. *kyushuensis*, subsp. *medellin*, and subsp. *jegathesan* were samples for DGGE. These products which were measured by agarose gel electrophoresis as the same size (438 bp) were separated based on their melting (denaturing) temperatures. PCR products of *B. thuringiensis* subsp. *israelensis* served as standards, since the *cry4A* and *cry4B* genes of *B. thuringiensis* subsp. *israelensis* are well documented. The standardized *cry4A* product migrated to a position during DGGE equivalent to a denaturant concentration of 53.5%. The standardized *cry4B* product migrated to a position in the denaturing gradient gel equivalent to 54.2% denaturant concentration. The *cry4B* products of *B. thuringiensis* subsp. *kyushuensis* stopped migration at 54.0% denaturant (Figure 4, Lane 2). The *cry4A* product of *B. thuringiensis* subsp. *medellin* stopped at 53.3% denaturant (Figure 4, Lane 3). The *cry4A* product of *B. thuringiensis* subsp. *jegathesan* stopped at 53.7% denaturant (Figure 4, Lane 4). Because of their unique positions during DGGE, *cry4* PCR products of *B. thuringiensis* subsp. *kyushuensis*, subsp. *medellin*, and subsp. *jegathesan* have at least one base change compared to standard *cry4* products. The *cry4* PCR products from *B. thuringiensis* subsp. *kyushuensis*, subsp. *medellin*, and subsp. *jegathesan* represent potential novel genes based on their unique melting temperatures.

DISCUSSION

DGGE was designed as a protocol for separating DNA fragments based on their sequences. The procedure has been used extensively in mutation detection. However, this is the first reported use of DGGE to find and identify novel genes. In this case, the size of PCR family products is small (438 bp), which is due to the locations of the primers within the genes. The small size of PCR products limits the application of RFLP (restriction fragment length polymorphism) as a tool to search for novel genes (Kuo and Chak 1996). DGGE becomes an alternative method for the identification. It has a high detection rate (50 - 70%) even without a GC clamp (Cotton 1997). When used for mutation detection, no false-positive

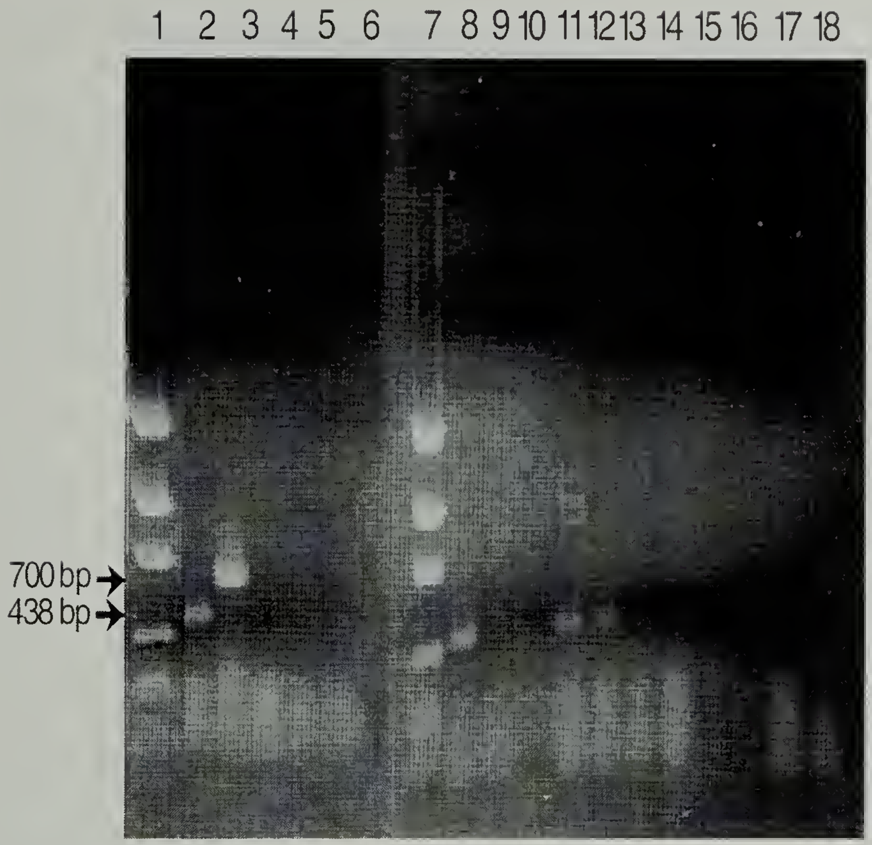

Figure 1. PCR cry4 family products (438 bp) amplified with cry4 family forward and reverse primers. Lanes 1 and 7. DNA molecular weight standards (top to bottom: 2.0, 1.2, 0.8, 0.4, 0.2, and 0.1 kb)

PCR products from template DNA from *B. thuringiensis* subspecies:

Lane 2. israelensis	Lane 11. medellin
Lane 3. canadensis	Lane 12. jegathesan
Lane 4. kenyae	Lane 13. fukuokaensis
Lane 5. aizawai	Lane 14. malaysiensis
Lane 6. morrisoni	Lane 15. darmstadiensis
Lane 8. kyushuensis	Lane 16. amagiensis
Lane 9. thompsoni	Lane 17. higo
Lane 10. neoleonensis	Lane 18. *E. coli*

Lanes 1-6 and Lanes 7-18 are from different gels.

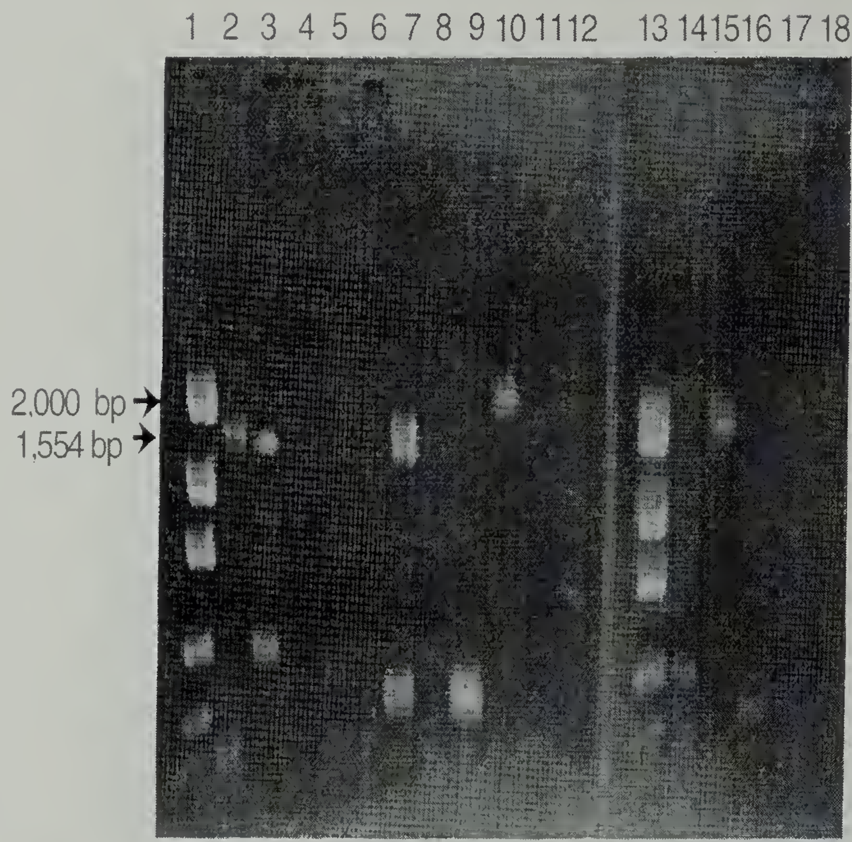

Figure 2. PCR cry4A type products (1554 bp) amplified with cry4A type forward primer and cry4 family reverse primer. Lanes 1 and 13. DNA molecular weight standards (top to bottom: 2.0, 1.2, 0.8, 0.4, 0.2, and 01 kb)

PCR products from template DNA from *B. thuringiensis* subspecies:
Lanes 2-6. Same as Figure 1.
Lane 7. kyushuensis
Lane 8. thompsoni
Lane 9. neoleonensis
Lane 10. medellin
Lane 11. jegathesan
Lane 12 fukuokaensis
Lane 14. malaysiensis
Lane 15. darmstadiensis
Lane 16. amagiensis
Lane 17. higo
Lane 18. *E. coli*
Lanes 1-12 and Lanes 13-18 are from different gels.

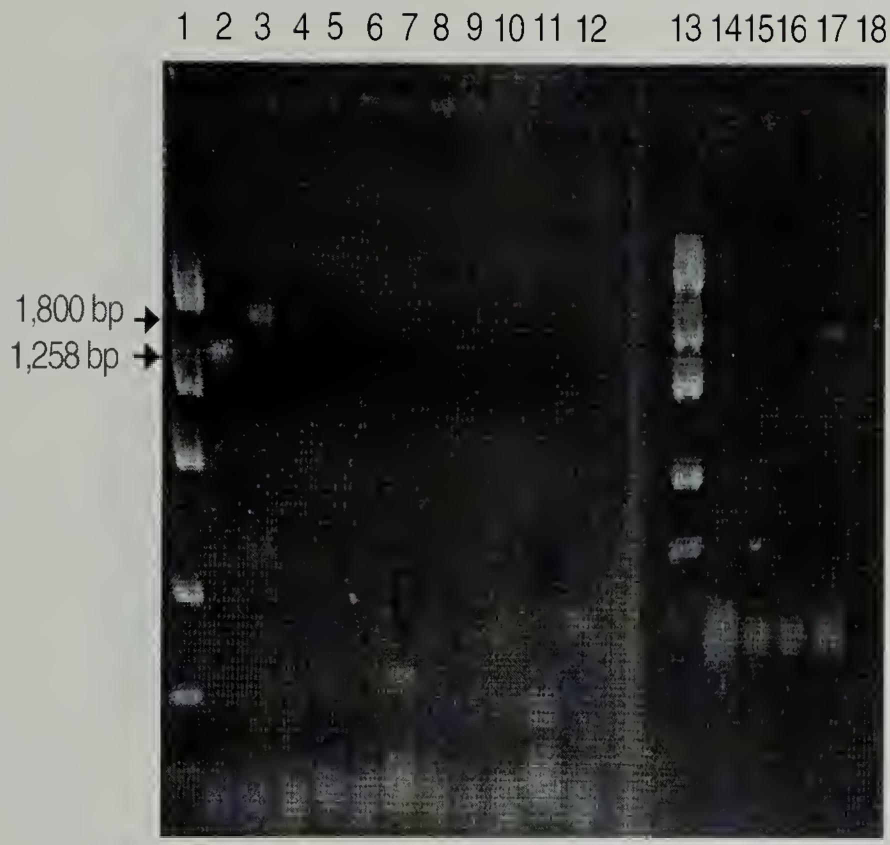

Figure 3. PCR cry4B type products (1,258 bp) amplified with cry4B type forward primer and cry4 family reverse primer. Lanes 1 and 13. DNA molecular weight standards (top to bottom: 2.0, 1.2, 0.8, 0.4, 0.2, 0.1 kb)

PCR products from template DNA from *B. thuringiensis* subspecies:
Lanes 2-6. Same as in Figure 1.
Lanes 8-16. Same as in Figure 2.
Lane 17. kyushuensis
Lane 18. *E. coli*
Lanes 1-12 and Lanes 13-18 all from different gels.

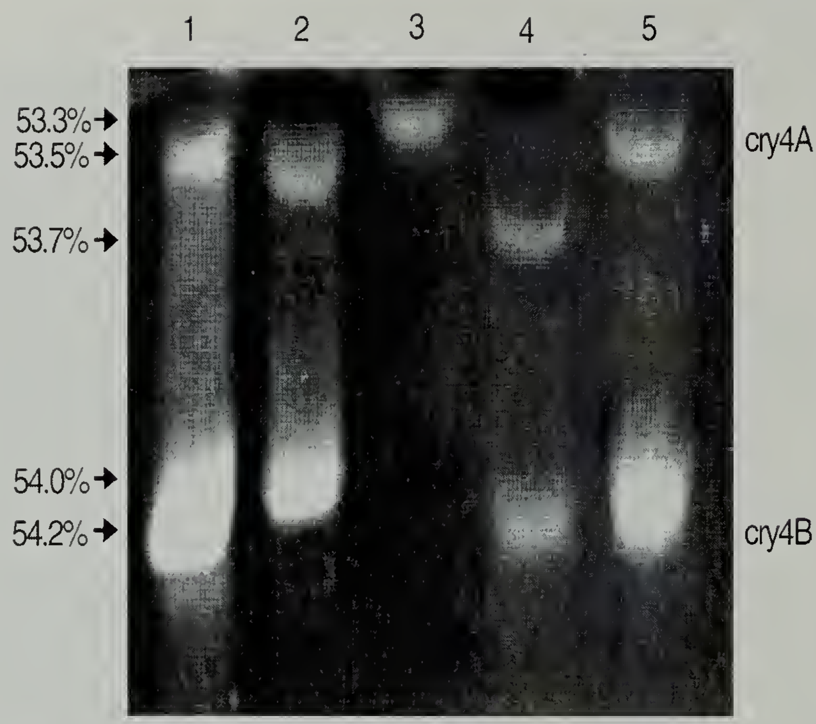

Figure 4. DGGE analysis of cry4 family products.

PCR cry4 family products from template DNA from *B. thuningiensis* subspecies:
Lane 1. israelensis
Lane 2. kyushuensis
Lane 3. medellin
Lane 4 jegathesan
Lane 5 israelensis

result has been reported in DGGE (Gejman et al. 1998). The high specificity makes DGGE effective in combination with E-PCR for specific gene identification.

In fact, DGGE method used in this study is highly sensitive. It can detect DNA sequences difference in only two base pairs. According to DNA sequences of PCR *cry4* family products amplified from *cry4A* and *cry4B* genes compared by BLAST program (NCBI), only two nucleic acids (T in position 224, and G in position 394) in *cry4A* fragment are switch to C and C, respectively, in *cry4B* fragment. These switches decrease the highest melting temperature of PCR *cry4* family products, amplified from *cry4A* gene, from 56.62° C to 55.83° C and increase the melting temperature in 5' end from 54.07° C to 54.44° C (Table 6). The changes of melting temperature caused the separation of *cry4A* and *cry4B* fragments of PCR *cry4* family products in denaturing gradient gel in this study.

Table 6. Melting temperature (Tm) (° C) and location within PCR family products

	cry4A	*cry4B*
Highest Tm	56.62	55.83
Location	middle	near 3'
Lowest Tm	54.04	54.09
Location	3'	5'
Tm at end	54.07	54.44
Location	5'	3'

When using *cry4A* specific primers in PCR, the product obtained from *B. thuringiensis* subsp. *medellin* was 2 kb, larger than the expected *cry4A* specific product of 1,554 bp. Because of the difference in observed and expected size, the *cry4A* product of *B. thuringiensis* subsp. *medellin* is from a potential novel gene. In the case of *B. thuringiensis* subsp. *jegathesan*, another novel *cry4* related gene is suspected. A PCR family product of expected size (438 bp) was observed when using *cry4* family forward and reverse primers. However, when using a *cry4A* or *cry4B* type forward primer paired with *cry4* family reverse primer in PCR, neither *cry4A* nor *cry4B* specific products were detected. These results were confirmed by DGGE. The match of DGGE results to verified PCR results indicates that DGGE results are predictable and reliable.

Because of the unique positions of the bands in DGGE gel, the PCR products of these bands have at least one base change altering their melting temperatures. Based on results of this study, it is not known whether the change in DNA sequence alters the amino acid sequence, because of degeneracy in the genetic code. Here, it is assumed that genes with at least a single base pair alternative are potential novel genes (Myers et al. 1985).

In this study, three novel genes within three strains were defined by their different locations in the linear DGGE gel. According to designations of reported *cry4* genes, the *cry4B*-related novel gene found from *B. thuringiensis* subsp. *kyushuensis* is named as *cry4Bb*. The *cry4A*-related novel gene found from *B. thuringiensis* subsp. *medellin* is named as *cry4Ab*. The *cry4A*-related novel gene found from *B. thuringiensis* subsp. *jegathesan* is named as *cry4Ac*. Further research, such as DNA sequencing, mRNA analysis, and protein

purification, is required to confirm the initial classifications and nomenclature of these novel genes. Protein analysis and amino acid sequencing also are required for further researches.

This is the first report of DGGE performed under conventional conditions. Based on previous reports, specific and expensive equipment and reagents are required for DGGE, which greatly limits its wide application. These requirements include GC-clamps, specific apparatus to hold the large size gel (17.5 cm wide x 20 cm long x 0.625 cm thick) and to maintain temperature (Myers, Manitatis, and Lerman 1987). In this study, a mini-gel (10 x 10 cm^2 with 1 mm thickness) was used. To achieve the same separation reported for large size gel, two mini-gels with different ranges of denaturant concentrations were used. The first gel had a wide range of denaturant (from 20% to 60%) and was used to determine the approximate position of each gene product. In the first gel, DNA banded at a range 52% to 55% denaturant concentrations. The denaturant concentration was narrowed for the second gel (from 50% to 60% denaturant) to resolve the bands. To maintain the running temperature, electrophoresis of DGGE gels were in an incubator with a small fan that maintained the air temperature at 60° C. The temperatures of running buffer in upper and lower tanks were the same as the air temperature. After 5 -6 hours run at 200 volts, no contoured results appeared, indicating that the gel did not overheat. Fragments of *cry4A* and *cry4B* were successfully separated from *cry4* family products under the conditions used in this study.

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PTERIDOPHYTES OF NORTHEAST ALABAMA AND ADJACENT HIGHLANDS IV: POLYPODIALES (Dryopteridaceae to Osmundaceae)

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INTRODUCTION

The following families are in the order Polypodiales treated in this paper are Dryopteridaceae (wood fern family), Hymenophyllaceae (filmy fern family), Lygodiaceae (climbing fern family), Marsileaceae (water-clover family), and Osmundaceae (royal fern family).

Information on specific and infraspecific taxa is set up in the following format: **Number.** *Name* author(s) [derivation of specific and infraspecific epithets]. VERNACULAR NAME. Habit; nativity (if exotic). Sporulating dates. Habitat data; highland provinces; relative abundance; [occurrence on Coastal Plain]. Conservation status. Wetland indicator status. Comments. *Synonyms.*

Introduced taxa are followed by a dagger (†). Species of conservation concern are followed by a star (★). The coded state ranks (ANHP 1994, 1996, 1997, 1999) are defined in Table 1. Wetland indicator status codes (Reed 1988) are defined in Table 2. Relative abundance is for occurrence in the study area and not for the whole state. Frequency of occurrence is defined as follows, ranging in descending order: *common* (occurring in abundance throughout), *frequent* (occurring throughout but not abundant), *occasional* (known in more than 50% of the region but in scattered localities), *infrequent* (known in less than 50% of t'

region in scattered localities or occurring in restricted habitats), *rare* (known from only a few counties and restricted to specific localities), and *very rare* (known from only a single or few populations; mostly narrow endemics, disjuncts, and peripheral taxa). Synonyms are from Mohr (1901)— M; Small (1938)— S; Radford *et al.* (1968)— R; and Lellinger (1985)— L. Suggested pronunciation, author(s), date of citation, common name, and derivations are provided after each genus.

Distribution maps are typically for 18 counties in the northeast region of Alabama. The maps are expanded to adjacent highland counties for taxa that are rare or peripheral. Key to symbols are as follows: Filled circle (•) = documented at Jacksonville State University herbarium; filled square (■) = documented at another herbarium; open circle (○) = reported in literature.

Table 1. Definition of state ranks.

Code	Definition
S1	*Critically imperiled* in Alabama because of extreme rarity or because of some factor(s) making it especially vulnerable to extirpation from Alabama.
S2	*Imperiled* in Alabama because of rarity or because of some factor(s) making it very vulnerable to extirpation from the state.
S3	*Rare or uncommon* in Alabama.
S4	*Apparently secure* in Alabama, with many occurrences.
S5	*Demonstrably secure* in Alabama and essentially "ineradicable" under present conditions.
SH	*Of historical occurrence*, perhaps not verified in the past 20 years, and suspected to be still extant.
SR	*Reported*, but without persuasive documentation which would provide a basis for either accepting or rejecting the report.
SU	*Possibly in peril* in Alabama, but status uncertain.
S?	*Not ranked* to date.

Table 2. Definition of wetland indicator codes.

Code	Status	Probability of Occurrence
OBL	Obligate Wetland Species	Occurs with estimated 99% probability in wetlands.
FACW	Facultative Wetland Species	Estimated 67%-99% probability of occurrence in wetlands, 1%- 33% probability in nonwetlands.
FAC	Facultative Species	Equally likely to occur in wetlands and nonwetlands(34%-66% probability).
FACU	Facultative Upland Species	Estimated 67%-99% probability of occurrence in nonwetlands, 1%-33% probability in wetlands.
UPL	Obligate Upland Species	Occurs with estimated 99% probability in uplands.
NI	No Indicator Status	Insufficient information available to determine an indicator status.

Note: Positive or negative signs indicate a frequency toward higher (+) or lower (-) frequency of occurrence within a category.

ORDER 2. POLYPODIALES (Continued)

5. Dryopteridaceae (Wood Fern Family)

Selected reference: Smith, A. R. 1993. Dryopteridaceae. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 246–249.

1. Leaves strongly dimorphic; sterile leaf blades pinnatifid and net veined, fertile leaf blades 2-pinnate and forming a bead-like cluster . . . . *Onoclea*
1. Leaves monomorphic, sterile and fertile leaf blades similar, not net-veined.
   2. Leaves 1-pinnate (leaflets not deeply lobed).

1. Leaves monomorphic, sterile and fertile leaf blades similar, not net-veined.
    2. Leaves 1-pinnate (leaflets not deeply lobed).
        3. Pinnae with anterior basal lobe, margins toothed; fertile pinnae at tips of leaves (apical); sori round . . . . *Polystichum*
        3. Pinnae lacking basal lobe, margins crenulate; fertile pinnae not localized at tip of leaves; sori elongate . . . . *Diplazium*
    2. Leaves pinnate-pinnatifid to 3-pinnate (leaflets deeply lobed or divided).
        4. Sori elongated or linear.
            5. Leaves 2-pinnate to 3-pinnate; sori crescent shaped . . . . *Athyrium*
            5. Leaves pinnate-pinnatifid; sori almost straight . . . . *Deparia*
        4. Sori round.
            6. Leaf stalk (petiole) essentially lacking scales (may have a few near base); indusia hood-like . . . . *Cystopteris*
            6. Leaf stalk distinctly scaly; indusia not hood-like.
                7. Larger pinnae mostly more than 5 cm long; rhizome robust; indusium covering top of sorus . . . . *Dryopteris*
                7. Larger pinnae mostly less than 5 cm long; rhizome slender; indusium covering sides of sorus (separating into flaps and forms a star-shaped cup) . . . . *Woodsia*

1. ATHYRIUM {eh-THIR-ee-um} Roth 1799 • Lady Ferns • [Greek *athyros*, doorless; sporangia tardily pushing open margin of indusium.]

Selected reference: Kato, M. 1993. *Athyrium*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 255–258.

1. *Athyrium filix-femina* (Linnaeus) Roth [lady-fern] var. ***asplenioides*** (Michaux) Farwell [like *Asplenium*, spleenwort]. SOUTHERN or LOWLAND LADY FERN. Figure 1. Deciduous perennial. Sporulates May – October. Low woods, wooded stream banks, swamps, moist sandstone bluffs, pond and lake margins; all highland provinces; common; [Coastal Plain]. Wetland Indicator Status, FAC. The specific epithet means lady-fern, referring to its delicate and lacy appearance. Native Americans used the rootstock to make a tea to help stop breast pains in child birth, induce lactation, and ease labor (Foster and Duke 1990). Synonyms: *Asplenium filix-foemina* (Linnaeus) Bernhardi— M; *Athyrium asplenioides* (Michaux) A. A. Eaton— S, R.

2. CYSTOPTERIS {sis-TOP-ter-iss} Bernhardi 1806 • Bladder Ferns • [Greek *kystos*, bladder, and *pteris*, fern; young indusia are inflated.]

Selected references: Blasdell, R. F. 1963. A monographic study of the fern genus *Cystopteris*. Mem. Torrey Bot. Club 21: 1–102. Haufler, C. H., R. C. Moran, and M. D. Windham. 1993. *Cystopteris*. In: Flora of North America Editorial Committee, eds.

1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 263-270. Haufler, C. H., M. D. Windham, and T. A. Ranker. 1990. Biosystematic analysis of the *Cystopteris tennesseensis* (Dryopteridaceae) complex. Ann. Missouri Bot. Gard. 77: 314-329.

1. Rhizome elongated (internodes distinct), apex usually protruding more than 1 cm past leaves; pinnules near middle of blade dissected to midrib (pinnate); bulblets absent; leaf blades usually widest near middle . . . . *C. protrusa*
1. Rhizome not elongated (internodes indiscernible due to closely overlappiing nodes), leaves clustered near apex; pinnules near middle of blade not dissected to midrib (pinnatifid); bulblets often present (found on the underside of leaf); leaf blades usually widest near base.
    2. Leaf apex gradually long-tapered (attenuate), often very elongated; gland-tipped hairs scattered along rachis and leaflets or absent; bulblets smooth, 2-3 mm in diameter and found on rachis and leaflets . . . . *C. bulbifera*
    2. Leaf apex not long-tapered (acute to acuminate), never greatly elongated; gland-tipped hairs absent or scarce; bulblets (when present) scaly, 1.5 mm in diameter or less and found only on rachis . . . . *C. tennesseensis*

1. ***Cystopteris bulbifera*** ★ (Linnaeus) Bernhardi [bearing bulbs]. Bulblet or Berry Bladder Fern. Figure 2. Deciduous perennial. Sporulates May - September. Shaded bluffs, often limestone; Interior Low Plateau, Cumberland Plateau; rare. State Rank, previously S? (ANHP 1994). Wetland Indicator Status, FAC. Propagation of new plants are often from bulblets found on the fronds, sometimes they develop new plants while still on the plant. Young fronds resemble *Cystopteris tennesseensis*.

2. ***Cystopteris protrusa*** (Weatherby) Blasdell [protruding]. Spreading Bladder Fern; Southern Fragile Fern; Lowland Brittle Fern. Figure 3. Deciduous perennial. Sporulates April - June. Rich, moist woods, often associated with limestone; Interior Low Plateau, Cumberland Plateau, Ridge and Valley; infrequent. Wetland Indicator Status, FACU. Plants spread by creeping rootstocks which protrude past the fronds, hence the specific epithet "*protrusa*." Synonym: *Cystopteris fragilis* (Linnaeus) Bernhardi var. *protrusa* Weatherby—M, S.

3. ***Cystopteris tennesseensis*** ★ Shaver [of Tennessee]. Tennessee Bladder Fern. Figure 4. Deciduous perennial. Sporulates April - August. Cracks and ledges of limestone cliffs and rocky limestone slopes; Interior Low Plateau, Cumberland Plateau; rare. State Rank, S2. Wetland Indicator Status, NI. This fern was discovered in 1950 by Jesse M. Shaver in Tennessee. It is a fertile hybrid derived from *Cystopteris protrusa* and *Cystopteris bulbifera*.

3. Deparia {dih-PAIR-ee-uh} Hooker & Greville 1830 • False Spleenworts • [Greek *depas*, saucer; from the saucer-like indusium of the type species *Deparia prolifera*.]

Selected reference: Kato, M. 1993. *Deparia*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York

and Oxford. Vol. 2, pp. 254–255.

1. ***Deparia acrostichoides*** (Swartz) M. Kato [like *Acrostichum*, leather fern]. SILVERY GLADE FERN; SILVERY-SPLEENWORT. Figure 5. Deciduous perennial. Sporulates June – September. Wooded limestone sinks and moist, rich woods, and rich slopes; all highland provinces; rare. Wetland Indicator Status, FAC. Young indusia have a silvery appearance. Synonyms: *Diplazium acrostichoides* (Swartz) Butters— S; *Athyrium thelypteroides* (Michaux) Desvaux— R, L.

4. DIPLAZIUM {dye-PLAY-zee-um} Swartz 1801 • Twin-sorus Ferns • [Greek *diplazein*, double; in reference to the sori being paired back-to-back in some species.]

Selected reference: Kato, M. 1993. *Diplazium*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 252–253.

1. ***Diplazium pycnocarpon*** ★ (Sprengel) M. Broun [with crowded "fruits"]. NARROW-LEAVED GLADE FERN; NARROW-LEAVED-SPLEENWORT. Figure 6. Deciduous perennial. Sporulates July – September. Wooded limestone sinks, moist woods and slopes in basic or neutral soils; all highland provinces; rare; [Coastal Plain]. Species of Special Concern (Freeman *et al.* 1979). Wetland Indicator Status, FAC. This fern was originally described by André Michaux in 1803 as a spleenwort because of its linear sori. Synonyms: *Asplenium angustifolium* Michaux— M; *Homalosorus pycnocarpus* (Sprengel) Small— S; *Athyrium pycnocarpon* (Sprengel) Tidestrom— R, L.

5. DRYOPTERIS {dry-OP-ter-iss} Adanson 1763 • Wood Ferns • [Greek, *drys*, tree (oak), and *pteris*, fern; an allusion to their woodland habitat.]

Selected references: Carlson, T. J. and W. H. Wagner, Jr. 1982. The North American distribution of the genus *Dryopteris*. Contr. Univ. Michigan Herb. 15: 141–162. Montgomery, J. D. and E. M. Paulton. 1981. *Dryopteris* in North America. Fiddlehead Forum 8: 25–31. Montgomery, J. D. and W. H. Wagner. 1993. *Dryopteris*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 280–289.

1. Leaf blades 3 to 4-pinnate; leaflets with bristle-like tips (spinulose toothed) . . . . D. intermedia
1. Leaf blades 1 to 2-pinnate, the pinnae often deeply cut (pinnatifid); leaflets lacking bristle-like tips.
   2. Sori near the margins of leaflets; indusia thick and swollen; leaves gray-green and leathery; plant of rocky, sloped woods and bluffs . . . . *D. marginalis*
   2. Sori not marginal; indusia thin and flat; leaves green and not leathery; plant of low, swampy woods and seepage slopes . . . . *D. celsa*

Note: *Dryopteris* X *australis* (Wherry) Small,★ SOUTHERN OR DIXIE WOOD FERN, an infertile hybrid of Log Fern, *Dryopteris celsa* and Florida Wood Fern, *Dryopteris ludoviciana* (Kunze) Small, has been extirpated from its type locality in Cherokee County, where it was originally discovered in 1936. An extant population is known from Lee County near Auburn (Wagner and Musselman 1982). Its State Rank is S1.

1. ***Dryopteris celsa*** ★ (W. Palmer) Small [high]. LOG FERN. Figure 7. Evergreen perennial. Sporulates June - September. Seepage slopes, low woods, and swamps; Interior Low Plateau, Cumberland Plateau, Ridge and Valley; rare. State Rank, S1. Wetland Indicator Status, FAC+. The specific epithet is referring to its habit of sometimes growing on logs, thus being high or exalted. This species is a fertile hybrid derived from Florida Wood Fern, *Dryopteris ludoviciana* (Kunze) Small, and Goldie's Wood Fern, *Dryopteris goldiana* (Hooker) Gray. Florida Wood Fern is found in swamps on the Coastal Plain of Alabama; Goldie's Wood Fern (erroneously reported for Alabama) is found further north of our state. Crested Wood Fern, *Dryopteris cristata* (Linnaeus) Gray, is a similar species and has been reported to occur in Alabama but was probably a misidentified Log Fern.

2. ***Dryopteris intermedia*** (Muhlenberg *ex* Willdenow) Gray [intermediate]. EVERGREEN or COMMON WOOD FERN; FANCY FERN. Figure 8. Evergreen perennial. Sporulates May - September. Rocky woods, often associated with hemlocks (*Tsuga*); Cumberland Plateau; rare. Wetland Indicator Status, FACU. Collected and used in floral arrangements, hence the name Fancy Fern. This species is similar to the Toothed Wood Fern, *Dryopteris carthusiana* (Villars) H. P. Fuchs [*Dryopteris spinulosa* (Mueller) Watt], which has been reported from the Bankhead National Forest in Winston County (Dean 1969), but no specimens have been seen. *Dryopteris intermedia* differs by having glandular blades and the 2 lower basal pinnules (closest to the rachis) usually of relatively the same size.

3. ***Dryopteris marginalis*** (Linnaeus) Gray [marginal]. MARGINAL SHIELD FERN; LEATHER WOOD FERN. Figure 9. Evergreen perennial. Sporulates May - September. Rocky, sloped woods, and sandstone bluffs; all highland provinces; frequent. Wetland Indicator Status, FACU. Sori are found on the margins of the pinnules, hence the specific epithet, *marginalis*. A sterile hybrid between *D. marginalis* and *D.intermedia* has been collected from Jackson County by Jack Short, who states that it is a striking and magnificent fern (Short 1999).

6. ONOCLEA {on-oh-KLEE-uh} Linnaeus 1753 • Sensitive Fern • [Greek *onos*, vessel, and *kleiein*, to close; sori are inclosed by inrolled leaf margins.] Monotypic genus, occurring in North America and northern Asia.

Selected references: Johnson, D. M. 1993. *Onoclea*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, p. 251. Lloyd, R. M. 1971. Systematics of Onocleoid ferns. Univ. Calif. Publ. Bot. 61: 1-86.

1. ***Onoclea sensibilis*** Linnaeus [sensitive]. SENSITIVE FERN; BEAD FERN. Figure 10. Deciduous perennial. Sporulates April - June. Marshes, low thickets, swamps, alluvial

woods, ditches, and creek banks; all highland provinces; common; [Coastal Plain]. Wetland Indicator Status, FACW. Sterile fronds are very "sensitive" to cold and will die with the first frost, leaving only the upright fertile frond with its bead-like sporophylls. This is the host plant for the Sensitive Fern Borer Moth (*Papaipema inquaesita*). This fern has caused poisonings in livestock. Animals that have ingested it developed symptoms of incoordination and are unable to walk or even eat temporarily (Gibbons *et al.* 1990). Not recommended for cultivation because of its aggressive nature in gardens.

7. POLYSTICHUM {poh-LISS-tik-um} Roth 1799 • Holly Ferns • [Greek, *poly*, many, *stichos*, rows; alluding to the sori on each pinna.] Vernacular name is in reference to the evergreen fronds and spinulose margins of the leaflets which resemble holly (*Ilex*).

Selected reference: Wagner, D. H. 1993. *Polystichum*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 290–299.

1. ***Polystichum acrostichoides*** (Michaux) Schott [like *Acrostichum*, leather fern]. CHRISTMAS FERN; DAGGER FERN. Figure 11. Evergreen perennial. Sporulates April - December. Moist woods and shaded slopes; all highland provinces; common; [Coastal Plain]. Wetland Indicator Status, FAC. Fronds stay green through the Christmas holiday season, hence the vernacular name. Used for Christmas wreaths and decorations. The leaflet with its "ear" at the base is thought to resemble a Christmas stocking or Santa in his sleigh. Cherokee Indians used its rhizomes as an ingredient in medicines for ailments such as toothaches and stomach aches (Dunbar 1989). This fern can be grown as a house plant and is often cultivated in moist shaded gardens. Various forms with rolled, ruffled or deeply incised leaflets are found growing naturally in Alabama.

8. WOODSIA {WOOD-zee-uh} R. Brown 1810 • Cliff Ferns • [For Joseph Woods, 1776–1864; English botanist.]

Selected references: Brown, D. F. M. 1964. A monographic study on the fern genus *Woodsia*. Nova Hedwigia 16: 1–154. Windham, M. D. 1993. *Woodsia*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 270–280.

1. ***Woodsia obtusa*** (Sprengel) Torrey [obtuse]. BLUNT-LOBED CLIFF FERN; COMMON WOODSIA. Figure 12. Deciduous perennial. Sporulates May - October. Moist, rocky woods, limestone bluffs, shaded sandstone cliffs, and sandstone outcrops; all highland provinces; frequent; [Coastal Plain]. Wetland Indicator Status, UPL. The specific epithet means "blunt" referring to the tips of the pinnules. This species is similar to *Cystopteris*, but can be distinguished by the numerous scales on the leaf stalk and the star-shaped sori.

6. HYMENOPHYLLACEAE (Filmy Fern Family)

Selected reference: Farrar, D. R. 1993. Hymenophyllaceae. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 190–191.

Note: *Hymenophyllum tayloriae* Farrar & Raine, ★ TAYLOR'S or GORGE FILMY FERN (see Figure 14), which primarily occurs only as a gametophyte, is known from Lawrence, Winston and Franklin counties in northwest Alabama. Its preferred habitat is deeply shaded, moist crevices in acidic rock, often narrow gorges and behind waterfalls. Gametophytes of *Hymenophyllum* are ribbon-like; whereas, gametophytes of *Trichomanes* are filamentous. Because of the preferred habitat and size of this species, it can be difficult to locate and to identify. Its State Rank is S1. Recent findings show that this species is likely to be more abundant than current herbarium records indicate (Davison 1997). Gametophytes of *Hymenophyllum tayloriae* were first discovered in 1936 by Mary S. Taylor in South Carolina. Sporophytes of this species were recently discovered growing with the gametophyte by Paul G. Davison in Lawrence County, Alabama, in the Bankhead National Forest (Farrar and Davison 1994).

1. TRICHOMANES {try-KAHM-uh-neez} Linnaeus 1753 • Bristle Ferns • [Greek *thrix*, hair, and *manes*, cup, alluding to the hair-like receptacle extending from the cup-like involucre that holds the sporangia.]

Selected references: Farrar, D. R., J. C. Parks, and B. W. McAlpin. 1982. The fern genera *Vittaria* and *Trichomanes* in the northeastern United States. Rhodora 85: 83–92. Farrar, D. R. 1993. *Trichomanes*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 226–227. Farrar, D. R. 1992. *Trichomanes intricatum*: The independent *Trichomanes* gametophyte in the eastern United States. Amer. Fern. J. 82: 83–92.

1. Plant filamentous, occurring as gametophyte only . . . . *T. intricatum*
1. Plant not filamentous, occurring as both gametophyte and sporophyte (gametophytes usually in association with sporophytes).
   2. Leaf blades entire to slightly lobed, usually less than 2 cm long; leaf margins with dark hairs . . . . *T. petersii*
   2. Leaf blades deeply pinnately lobed, usually greater than 4 cm long; leaf margins without dark hairs . . . . *T. boschianum*

1. *Trichomanes boschianum* ★ Sturm [R. B. Van den Bosch, 1810–1862]. APPALACHIAN FILMY FERN; BRISTLE FERN. Figure 13. Deciduous perennial. Sporulates June – September (sexual reproduction is thought to be infrequent). Moist shaded areas such as rock overhangs, deep shelters or grottoes, that usually are not exposed to climatic extremes; Cumberland Plateau, Ridge and Valley, upper Piedmont; rare. State Rank, S3 (ALHP 1994). Wetland

Indicator Status, OBL (probably FAC). This species was first discovered in Alabama along the Sipsey River by Judge Thomas Peters in 1853 (Mohlenbrock and Voigt 1959). The specific epithet commemorates Roelof van den Bosch, a nineteenth century botanist who studied filmy ferns.

2. ***Trichomanes intricatum*** Farrar [entangled]. WEFT FERN. Figure 14. Gametophyte only. Deeply sheltered overhangs of sandstone rocks; Cumberland Plateau, Ridge and Valley; infrequent. Wetland Indicator Status, NI. Because this species is difficult to locate (due to habit) and to identify, the frequency of occurrence in the study area is underestimated. Dr. Herb Wagner says it looks like "green steel-wool."

3. ***Trichomanes petersii*** ★ A. Gray [T. M. Peters, ?-1888]. DWARF FILMY FERN; PETERS' BRISTLE FERN. Figure 15. Deciduous perennial. Sporulates June - August. Moist shaded areas such as rock faces, ledges, and sheltered rocky areas, often close to waterfalls; Cumberland Plateau, Ridge and Valley, Piedmont Plateau; rare; [Coastal Plain]. State Rank, S2. Wetland Indicator Status, FAC. Asa Gray named this species in honor of Judge Thomas M. Peters a graduate of the University of Alabama., who first discovered it in 1853 in Winston County, Alabama (Thieret 1980). The type locality of this fern was destroyed when the Lewis Smith Dam was build on the Sipsey River (Dean 1969).

7. LYGODIACEAE (Climbing Fern Family)

1. LYGODIUM {lye-GO-dee-um} Swartz 1800 • Climbing Ferns • [Greek *lygodes*, flexible, in reference to the twining habit.] The "leaves" of this genus are leaflets (pinnules) and the true leaves are the whole vine-like frond, of indeterminate growth. This genus (and family) is sometimes included in the Schizaeaceae (Curly-grass Family).

Selected reference: Nauman, C. E. 1993. Lygodiaceae. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. New York and Oxford. Vol. 2, p. 114–116.

1. Leaflets ("leaves") palmately lobed and fan-shaped; sterile tissue between indusia on fertile lobes nearly absent . . . . *L. palmatum*
1. Leaflets ("leaves") 1- to 3-pinnate, not fan-shaped; sterile tissue between indusia on fertile lobes present . . . . *L. japonicum*

1. ***Lygodium japonicum*** † (Thunberg *ex* Murray) Swartz [Japanese]. JAPANESE CLIMBING FERN. Figure 16. Deciduous, vine-like perennial; native to eastern Asia. Sporulates June - September. Roadside ditches, creek banks, moist woods; Cumberland Plateau, Ridge and Valley; rare; [chiefly Coastal Plain]. Wetland Indicator Status, FAC. This fern has been grown in outdoor gardens throughout the South and was first reported to have escaped from cultivation in Thomasville, Georgia in the early 1900's (Nelson 2000). In our area, it is only sporadically naturalized, but is a rampant weed in southern Alabama and other southeastern states.

2. ***Lygodium palmatum*** ★ (Bernhardi) Swartz [hand-like]. AMERICAN CLIMBING FERN;

HARTFORD FERN. Figure 17. Deciduous, vine-like perennial. Sporulates June - September. Wet thickets in sandy or acid soil; Cumberland Plateau; very rare; [Coastal Plain]. State Rank, S1. Wetland Indicator Status, FACW. One known extant population in northeast Alabama, is found below the confluence of the east and west forks of Little River on Cherokee-DeKalb County line. A report of this species exists for Jackson County (Short 1978). This fern was once common around Hartford, Connecticut, (hence the name), and because of over-collecting, the State Legislature passed a law to protect this fern from being taken from another person's property (Clute 1938).

8. MARSILEACEAE (Water-clover Family)

Selected reference: Johnston, D. M. 1993. Marsileaceae. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 331-335.

1. PILULARIA {pill-yoo-LARE-ee-uh} Linnaeus 1754 • Pillworts • [Latin *pilula*, referring to the tiny ball-like sporocarps.]

1. *Pilularia americana* ★ A. Braun [American]. AMERICAN PILLWORT. Figure 18. Evergreen, aquatic. Sporocarps produced April - October. Shallow water of lakes, ponds, and streams in the Tennessee River Valley in the Interior Low Plateau and Cumberland Plateau; rare. State Rank, S1. Wetland Indicator Status, OBL. The little "pills" which contain the spores allow the plant to survive droughts (Dean 1969). The best characteristic to distinguish this fern from other grass-like plants are by its curled up leaf tips ("fiddleheads") that will uncoil as the leaves mature. Only one herbarium specimen seen from Alabama. It was collected by Dr. Paul Davison, in Lauderdale County near Waterloo.

9. OSMUNDACEAE (Royal Fern Family)

Selected references: Bobrow, A. E. 1967. The family Osmundaceae (R. Br.) Kaulf. its taxonomy and geography. Bot. Zhurn. Moscow & Leningrad 52: 1600-1610. Hewitson, W. 1962. Comparative morphology of the Osmundaceae. Ann. Missouri Bot. Gard. 49: 57-93. Whetstone, R. D. and T. A. Atkinson. 1993. Osmundaceae. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 107-109.

1. OSMUNDA {oz-MUN-da} Linnaeus 1753 • Royal Ferns • [Saxon, *Osmunder*, name for Thor, God of war; the type species, *O. regalis*, grew in bogs where bog iron was found; the ore was used to make weapons (and other items).]

1. Sterile leaves pinnate, leaflets pinnatifid (deeply lobed); tufts of hairs persistent on abaxial surface (underside) of pinnae (leaflets) near base; fertile leaves with pinnae monomorphic, all spore bearing . . . . *O. cinnamomea*

1. Sterile leaves 2-pinnate; tufts of hairs absent on abaxial surface of pinnae near base; fertile leaves with pinnae dimorphic, apical ones spore-bearing, the others not . . . *O. regalis*

1. *O. cinnamomea* Linnaeus [cinnamon-colored]. CINNAMON FERN. Figure 19. Deciduous perennial. Sporulates March – May. Seepage woods, bogs, swamps, stream banks, and moist sandstone bluffs; all highland provinces; frequent; [Coastal Plain]. Wetland Indicator Status, FACW+. Mature fertile fronds are covered with cinnamon colored hairs, hence the common name. Widely cultivated as an ornamental. This and the next species are host plants for the Osmunda Borer Moth (*Papaipema speciosissima*). Cherokee Indians ate new fiddle-heads (croziers) as a vegetable (Dunbar 1989). The rhizome is edible and is reported to taste like raw cabbage (Clute 1938).
2. *O. regalis* Linnaeus [royal] var. ***spectabilis*** (Willdenow) Gray [showy]. ROYAL FERN. Figure 20. Deciduous perennial. Sporulates March – June. Seepage woods, bogs, stream banks, and swamps; all highland provinces; infrequent; [Coastal Plain]. Wetland Indicator Status, OBL. Rhizomes are used by orchid growers. Fiber from the rhizome is also used to make twine, rope, netting, and mats (Dunbar 1989). The chloroplasts within the spores give the young sporangia their green color. As the spores mature and are shed, the sporangia change color to a distinctive, rusty brown. The white portion of the rhizome is edible; because of its pungent taste is has been called bog onion (Abbe 1981). The type species of this very large and regal fern (*O. regalis* var. *regalis*) occurs in Eurasia.

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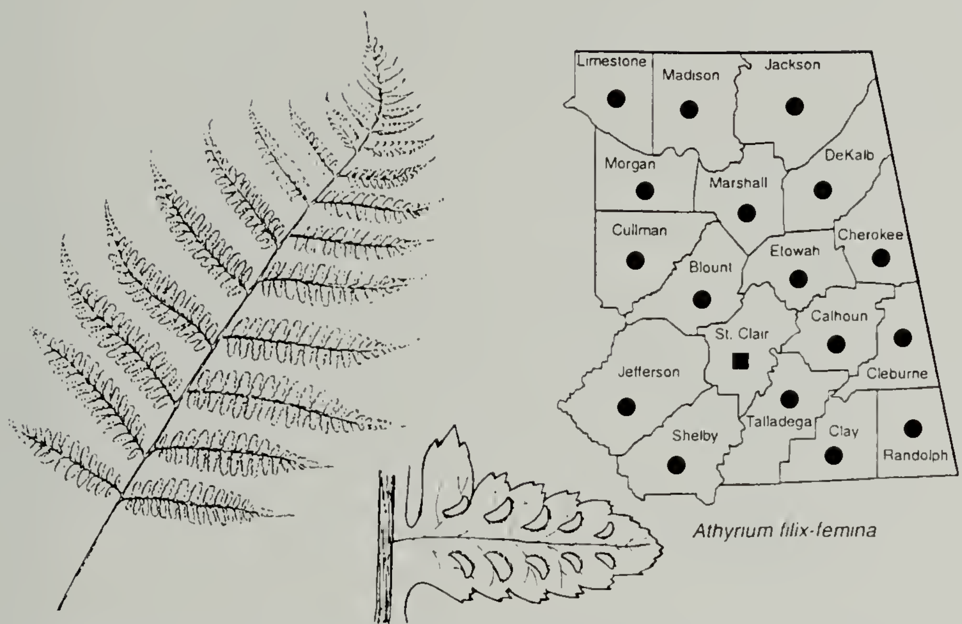

Figure 1. *Athyrium filix-femina*- Southern Lady Fern

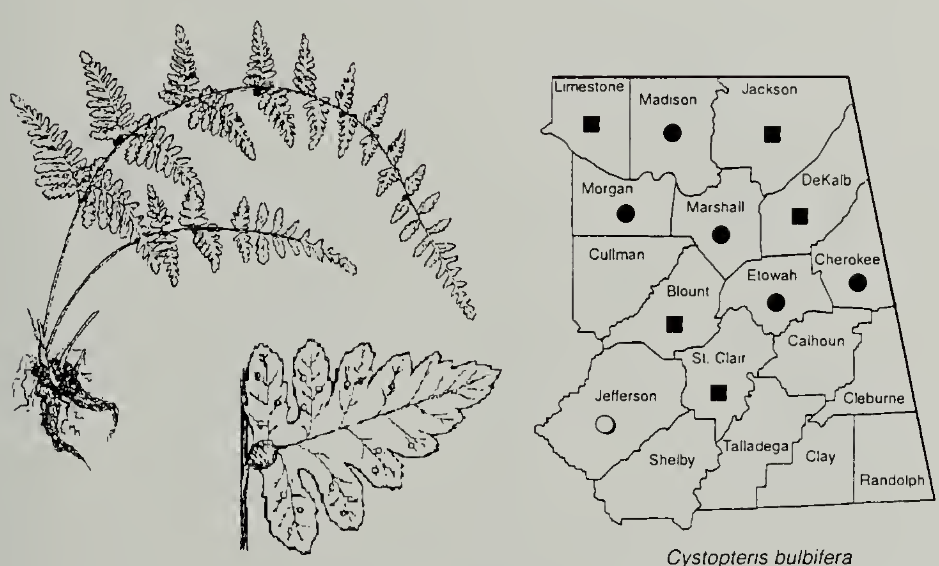

Figure 2. *Cystopteris bulbifera*- Bulblet Bladder Fern

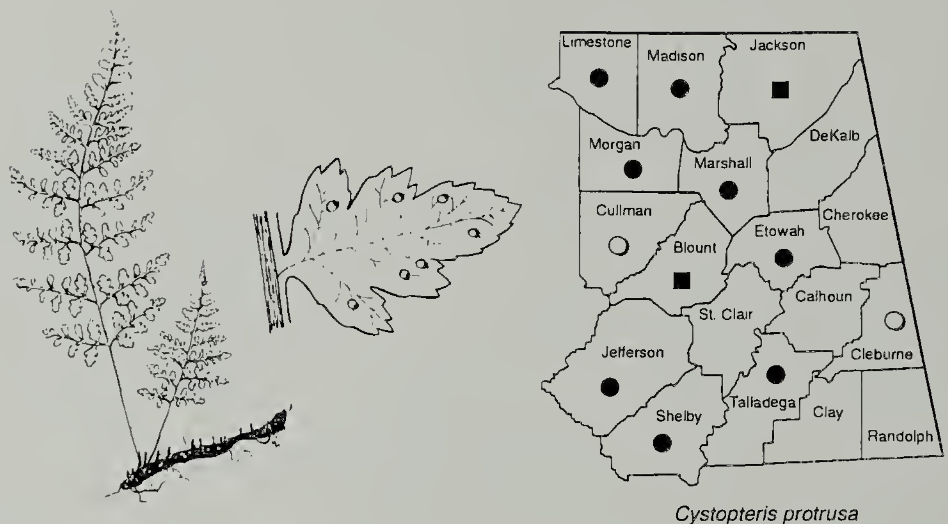

Figure 3. *Cystopteris protrusa*- Spreading Bladder Fern

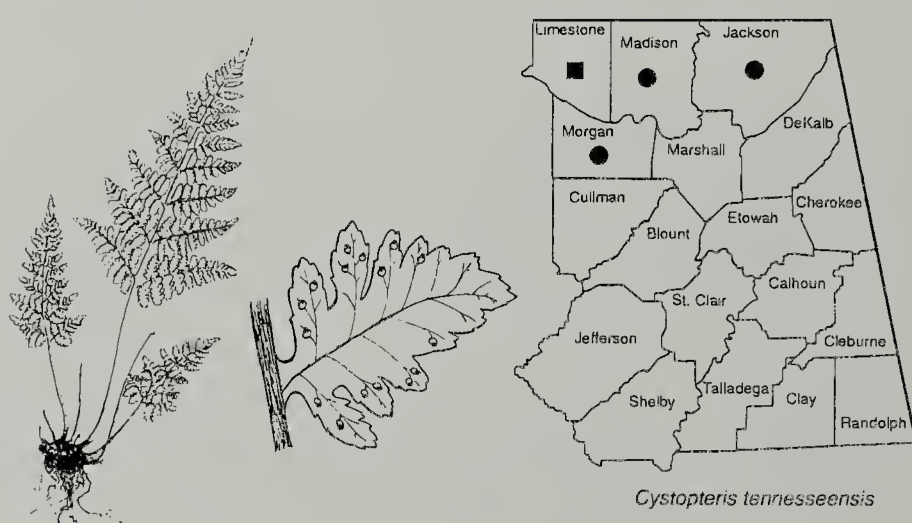

Figure 4. *Cystopteris tennesseenis*- Tennessee Bladder Fern

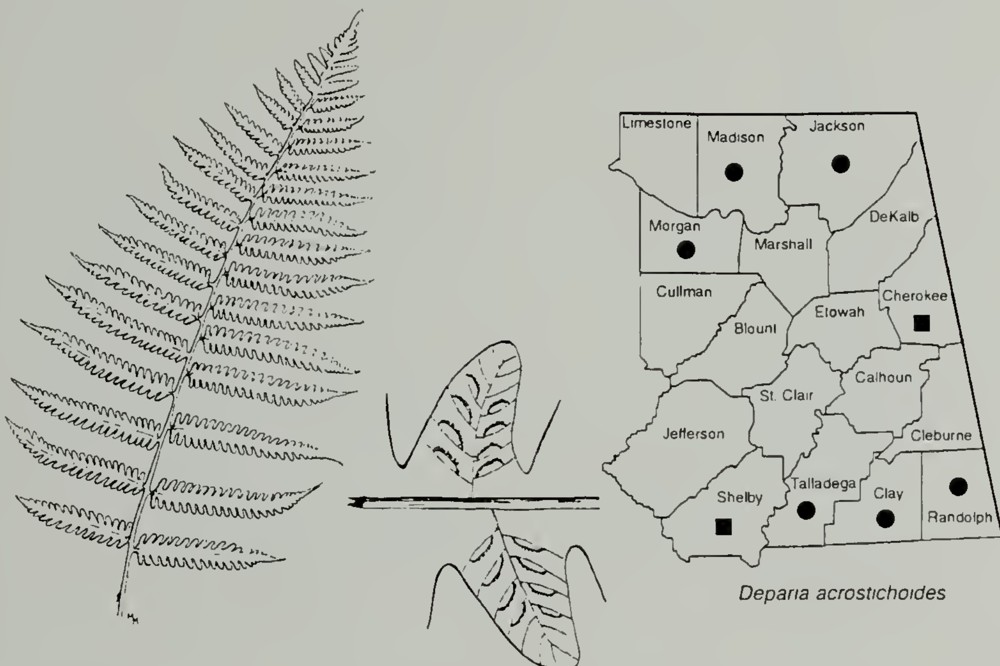

Figure 5. *Deparia acrostichoides*- Silvery Glade Fern

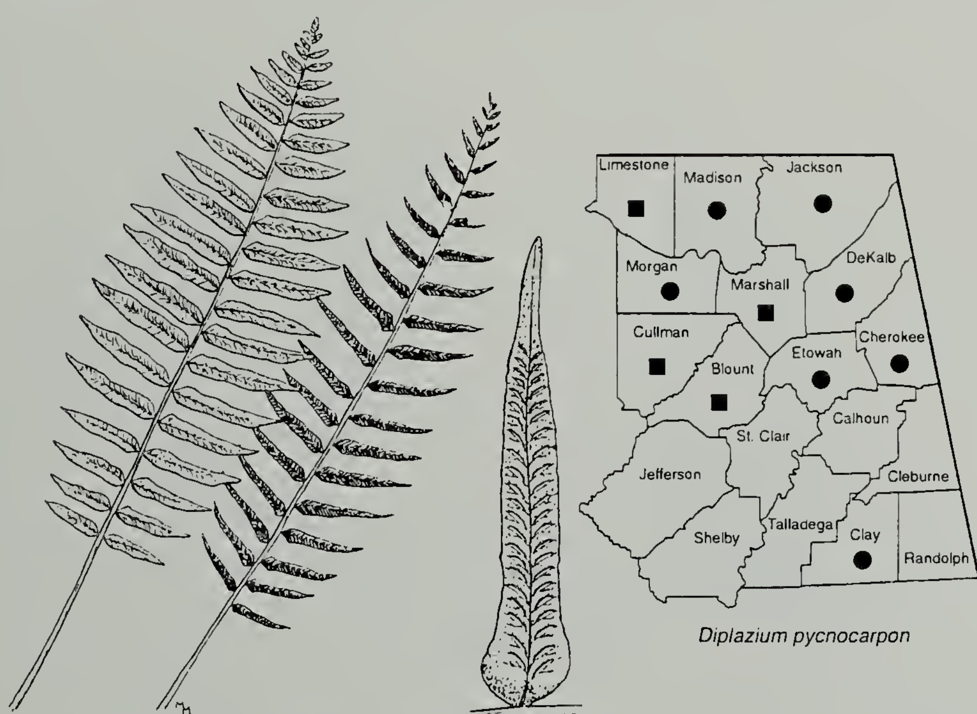

Figure 6. *Diplazium pycnocarpon*- Narrow-leaved Glade Fern

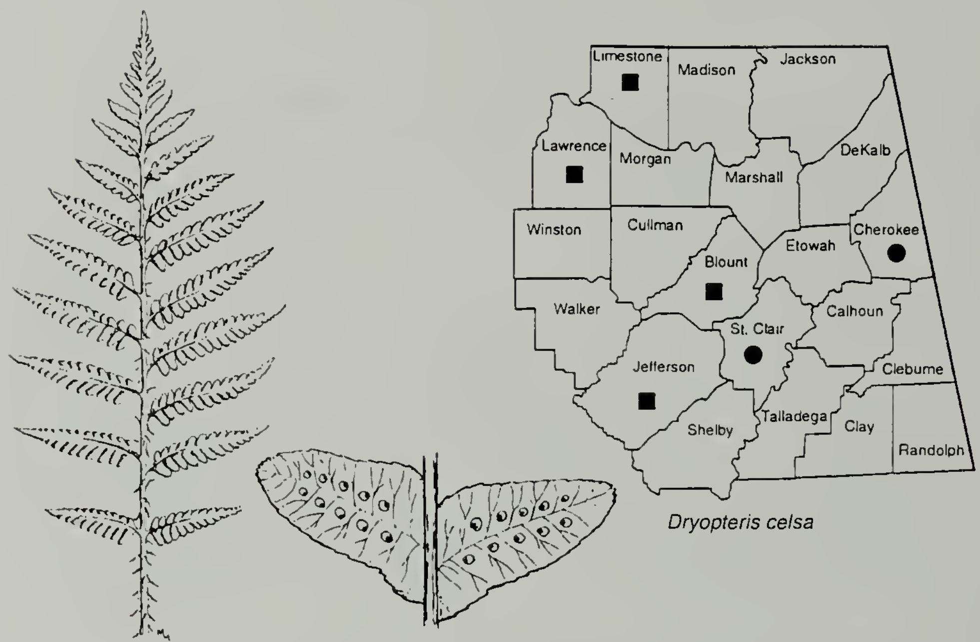

Figure 7. *Dryopteris celsa*- Log Fern

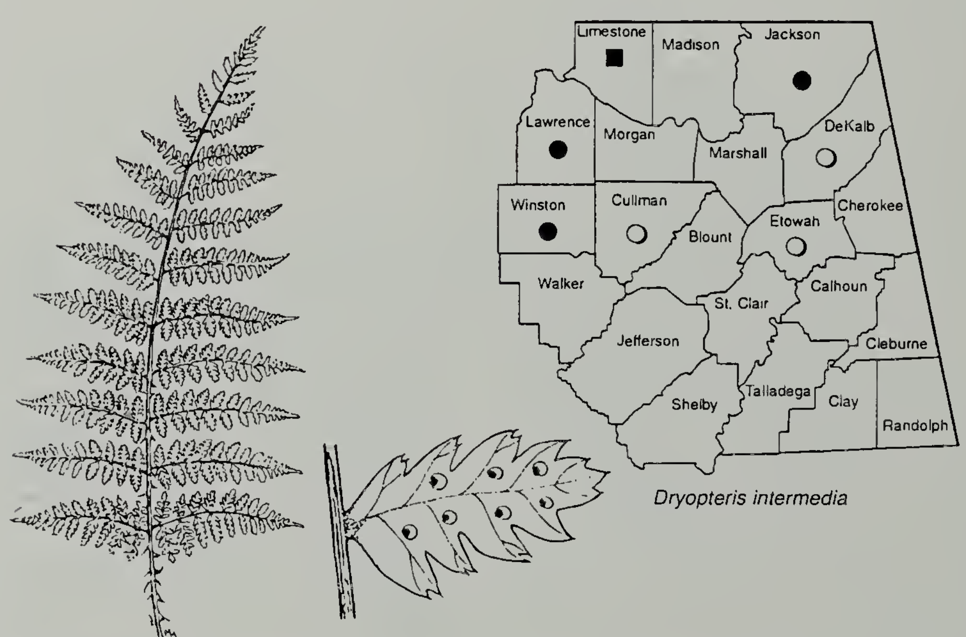

Figure 8. *Dryopteris intermedia*- Evergreen Wood Fern

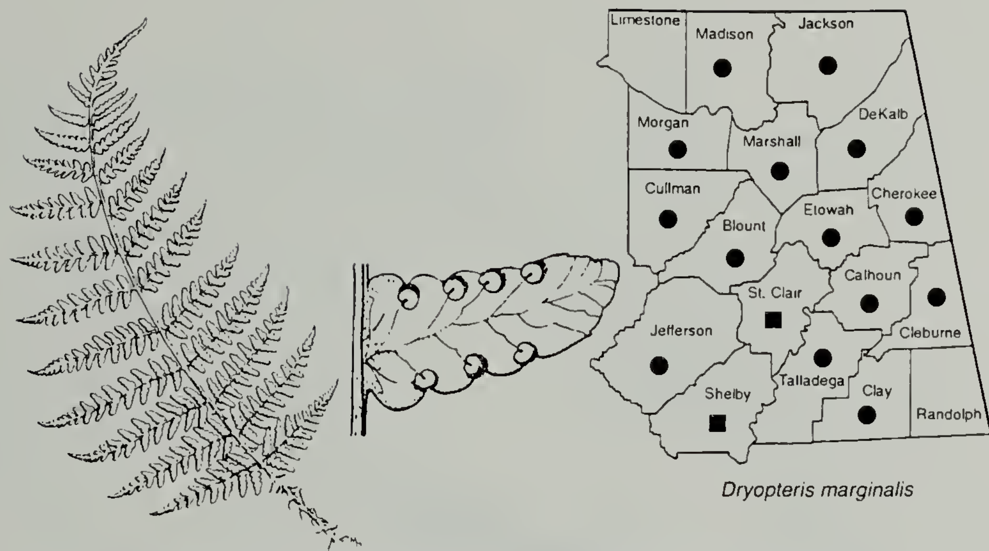

Figure 9. *Dryopteris marginalis*- Marginal Shield Fern

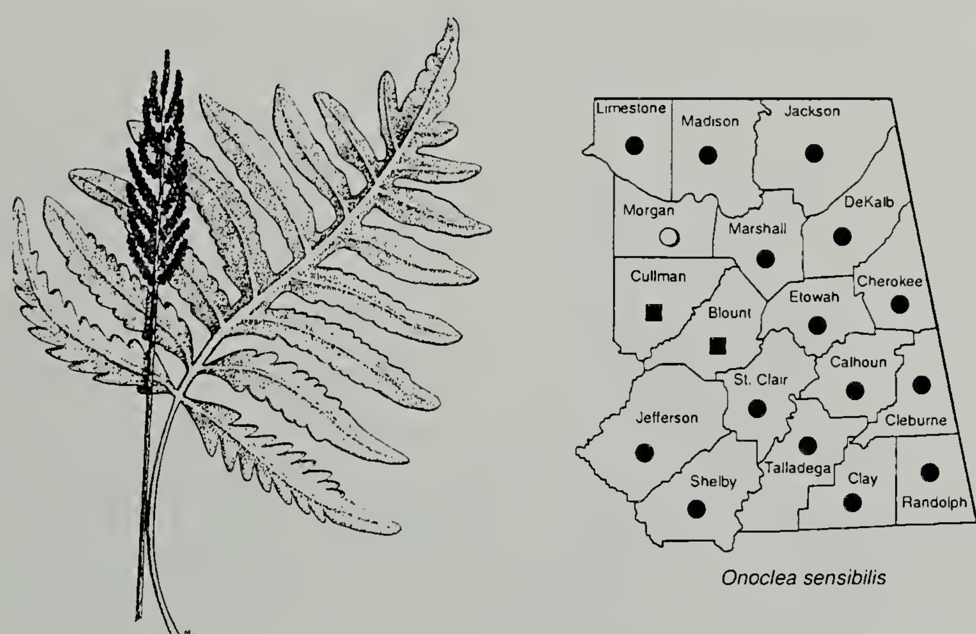

Figure 10. *Onoclea sensibilis*- Sensitive Fern

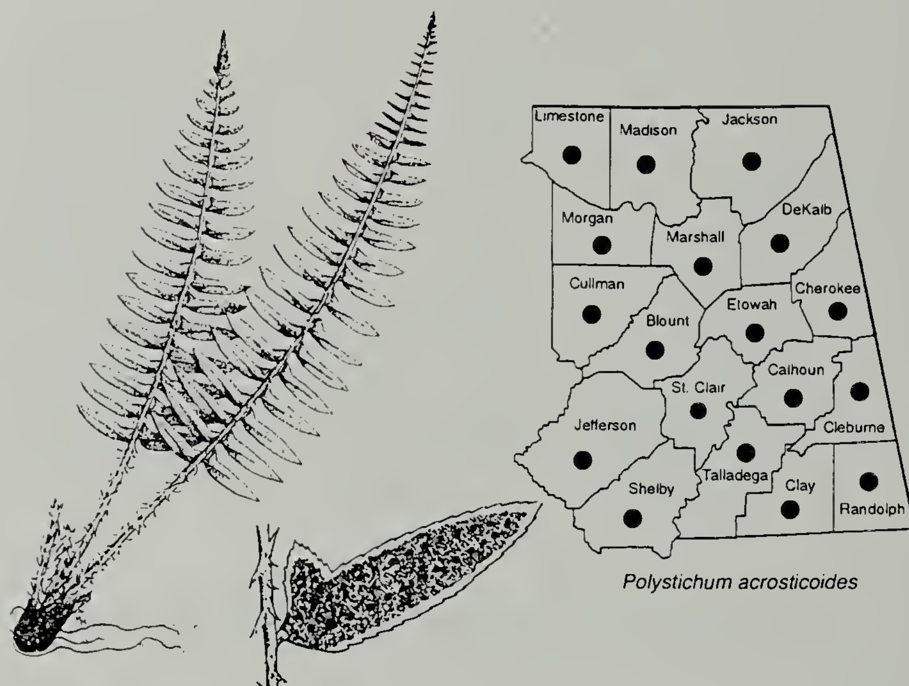

Figure 11. *Polystichum acrostichoides-* Christmas Fern

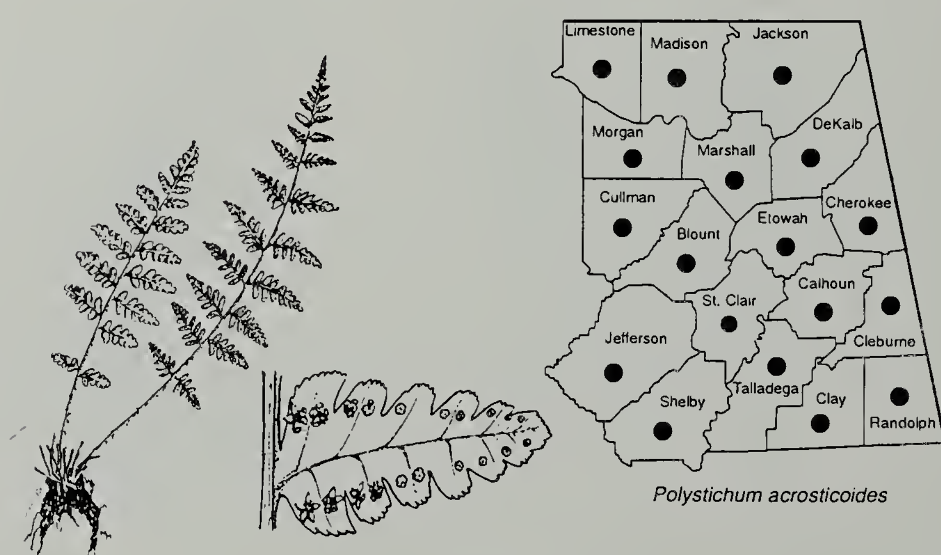

Figure 12. *Woodsia obtusa-* Blunt-lobed Cliff Fern

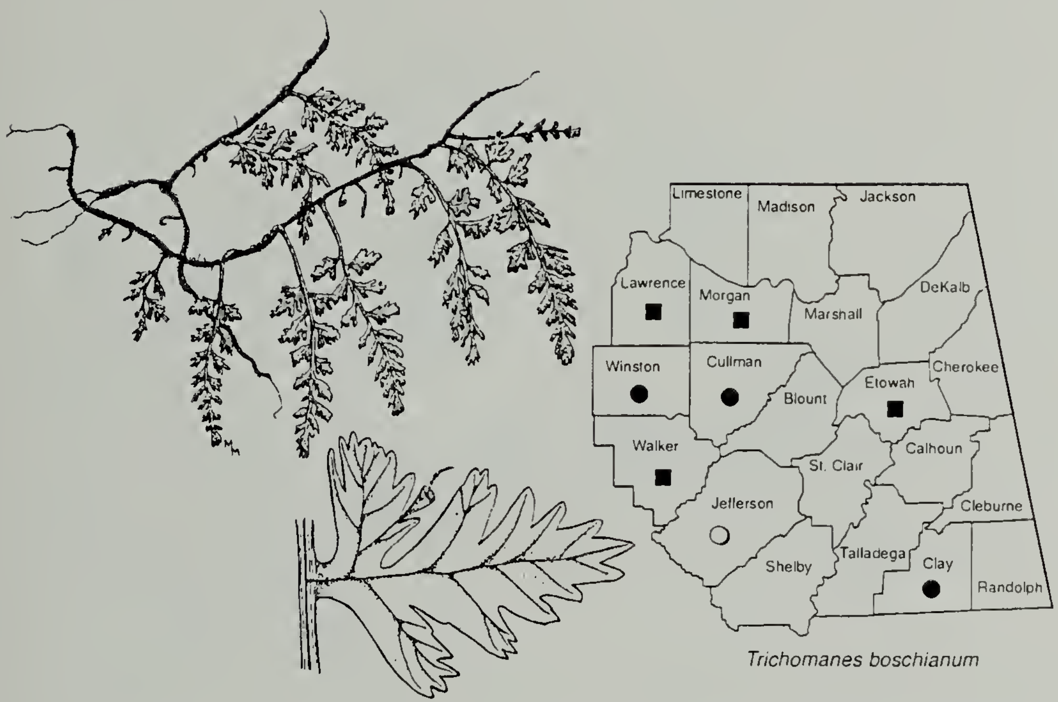

Figure 13. *Trichomanes boschianum*- Appalachian Filmy Fern

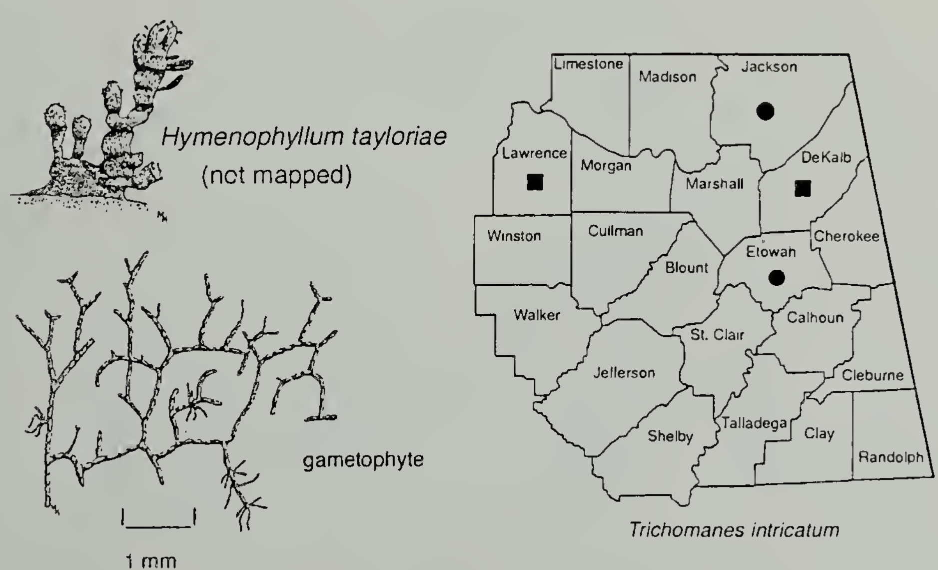

Figure 14. *Trichomanes intricatum*- Weft Fern

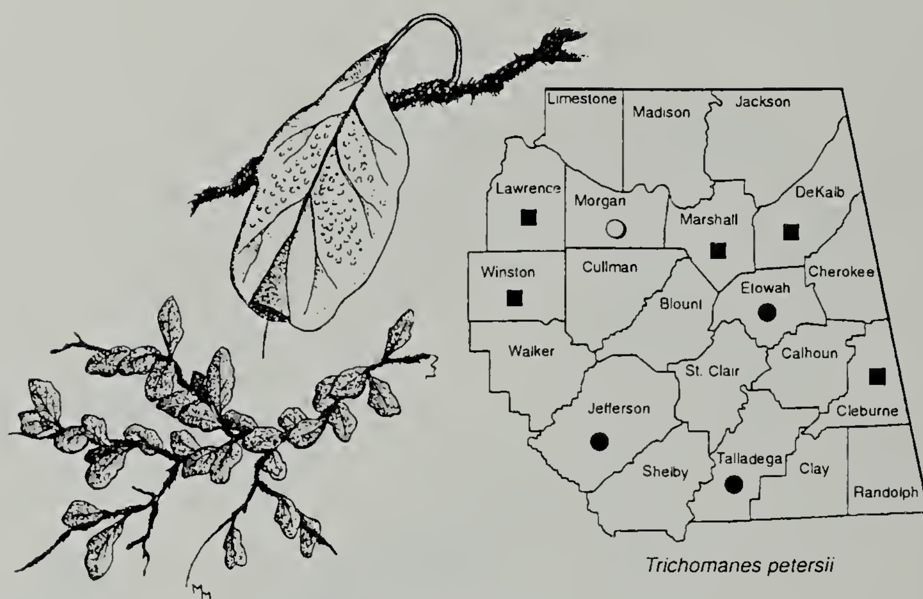

Figure 15. *Trichomanes petersii*- Dwarf Filmy Fern

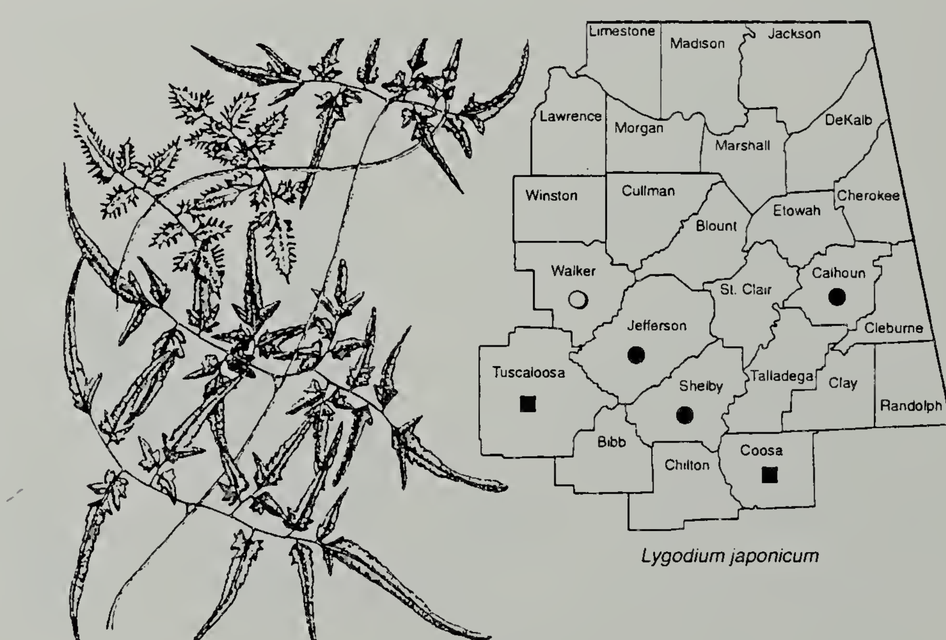

Figure 16. *Lygodium japonicum*- Japanese Climbing Fern

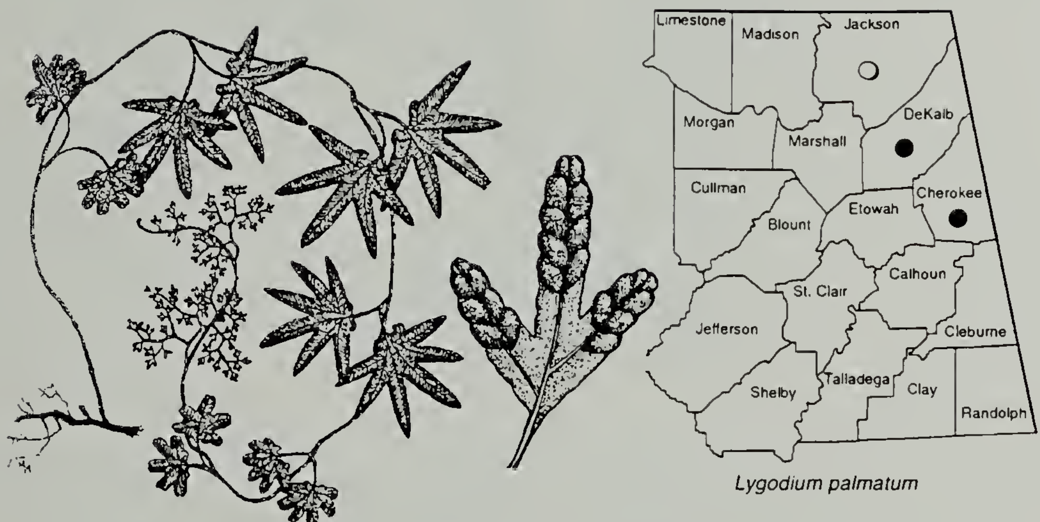

Figure 17. *Lygodium palmatum*- American Climbing Fern

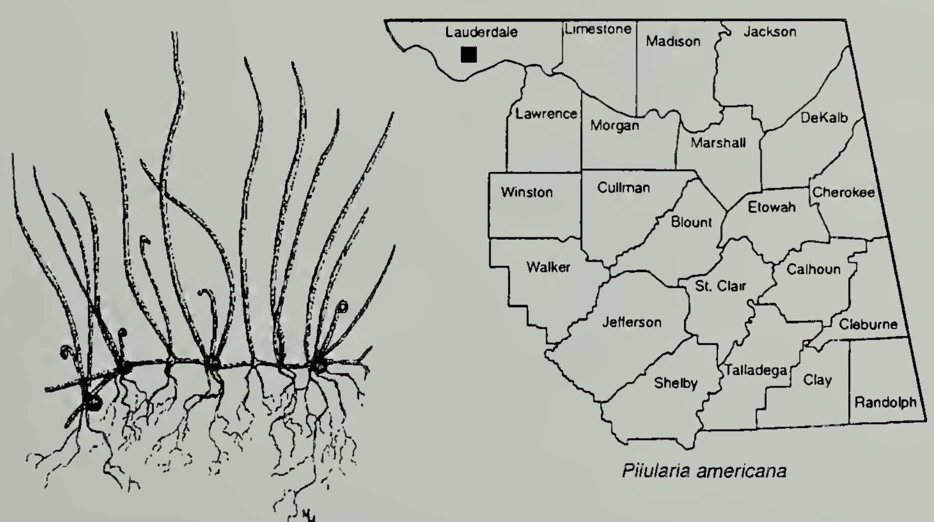

Figure 18. *Pilularia americana*- American Pillwort

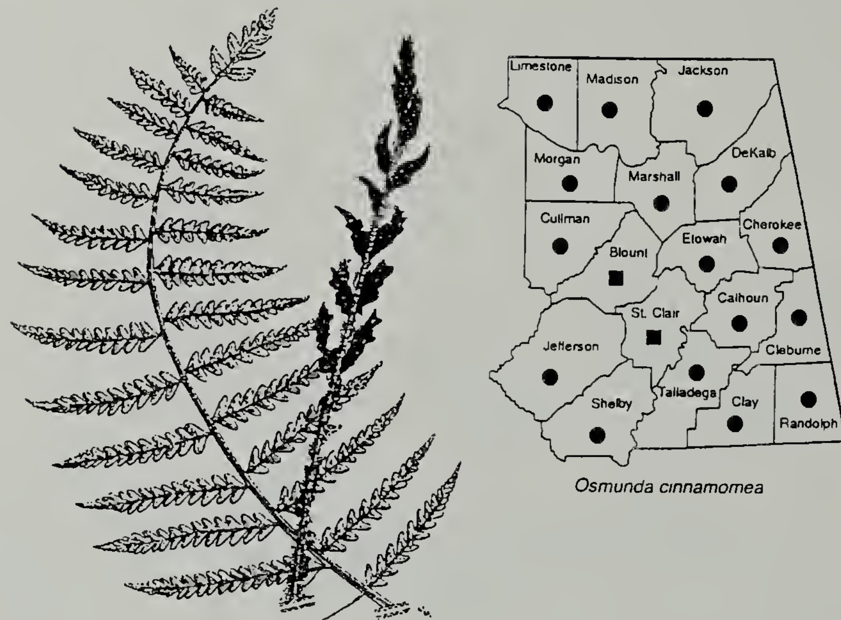

Figure 19. *Osmunda cinnamomea*- Cinnamon Fern

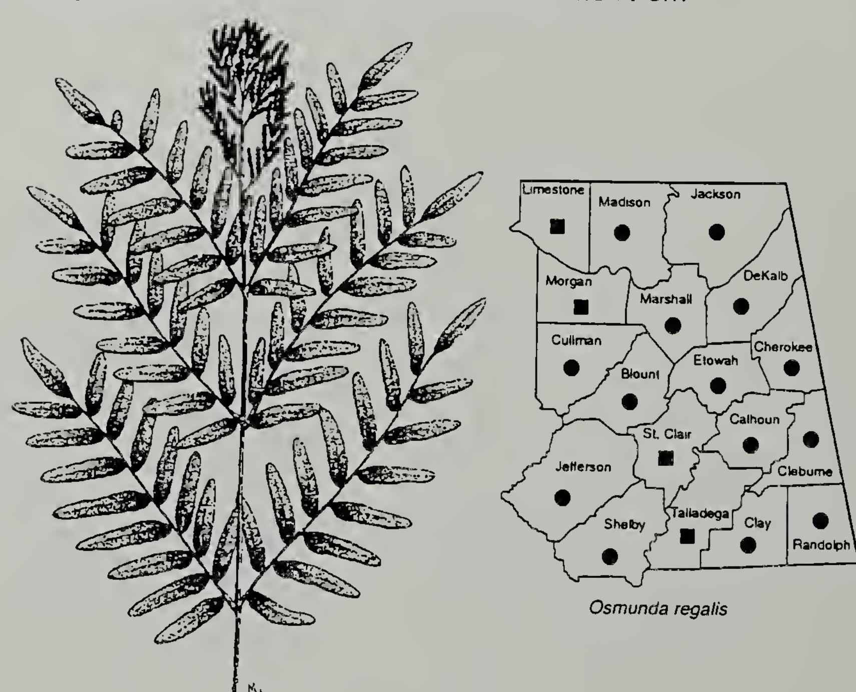

Figure 20. *Osmunda regalis*- Royal Fern

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PTERIDOPHYTES OF NORTHEAST ALABAMA AND ADJACENT HIGHLANDS V: POLYPODIALES (Polypodiaceae to Vittariaceae)

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INTRODUCTION

This paper concludes the study of pteridophytes of Northeast Alabama and adjacent highlands. The final families treated are Polypodiaceae (polypody family), Pteridaceae (maidenhair fern family), Thelypteridaceae (marsh fern family), and Vittariaceae (shoestring fern family).

Information on specific and infraspecific taxa is set up in the following format: **Number.** ***Name*** author(s) [derivation of specific and infraspecific epithets]. VERNACULAR NAME. Habit; nativity (if exotic). Sporulating dates. Habitat data; highland provinces; relative abundance; [occurrence on Coastal Plain]. Conservation status. Wetland indicator status. Comments. *Synonyms.*

Introduced taxa are followed by a dagger (†). Species of conservation concern are followed by a star (★). The coded state ranks (ANHP 1994, 1996, 1997, 1999) are defined in Table 1. Wetland indicator status codes (Reed 1988) are defined in Table 2. Relative abundance is for occurrence in the study area and not for the whole state. Frequency of occurrence is defined as follows, ranging in descending order: *common* (occurring in abundance throughout), *frequent* (occurring throughout but not abundant), *occasional* (known in more than 50% of the region but in scattered localities), *infrequent* (known in less than 50% of the

region in scattered localities or occurring in restricted habitats), *rare* (known from only a few counties and restricted to specific localities), and *very rare* (known from only a single or few populations; mostly narrow endemics, disjuncts, and peripheral taxa). Nomenclature follows Flora of North America [FNA] (1993+) and more recent publications. Synonyms are from Mohr (1901)— M; Small (1938)— S; Radford *et al.* (1968)— R; and Lellinger (1985)— L. Suggested pronunciation, author(s), date of citation, common name, and derivations are provided after each genus.

Distribution maps are typically for 18 counties in the northeast region of Alabama. The maps are expanded to adjacent highland counties for taxa that are rare or peripheral. Key to symbols are as follows: Filled circle (•) = documented at Jacksonville State University herbarium; filled square (■) = documented at another herbarium; open circle (○) = reported in literature.

Table 1. Definition of state ranks.

Code	Definition
S1	*Critically imperiled* in Alabama because of extreme rarity or because of some factor(s) making it especially vulnerable to extirpation from Alabama.
S2	*Imperiled* in Alabama because of rarity or because of some factor(s) making it very vulnerable to extirpation from the state.
S3	*Rare or uncommon* in Alabama.
S4	*Apparently secure* in Alabama, with many occurrences.
S5	*Demonstrably secure* in Alabama and essentially "ineradicable" under present conditions.
SH	*Of historical occurrence*, perhaps not verified in the past 20 years, and suspected to be still extant.
SR	*Reported*, but without persuasive documentation which would provide a basis for either accepting or rejecting the report.
SU	*Possibly in peril* in Alabama, but status uncertain.
S?	*Not ranked* to date.

Table 2. Definition of wetland indicator codes.

Code	Status	Probability of Occurrence
OBL	Obligate Wetland Species	Occurs with estimated 99% probability in wetlands.
FACW	Facultative Wetland Species	Estimated 67%-99% probability of occurrence in wetlands, 1%- 33% probability in nonwetlands.
FAC	Facultative Species	Equally likely to occur in wetlands and nonwetlands (34%-66% probability).
FACU	Facultative Upland Species	Estimated 67%-99% probability of occurrence in nonwetlands, 1%-33% probability in wetlands.
UPL	Obligate Upland Species	Occurs with estimated 99% probability in uplands.
NI	No Indicator Status	Insufficient information available to determine an indicator status.

Note: Positive or negative signs indicate a frequency toward higher (+) or lower (-) frequency of occurrence within a category.

ORDER 2. POLYPODIALES (Continued)

10. Polypodiaceae (Polypody Family)

Selected reference: Smith, A. R. 1993. *Polypodiaceae*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 312-313.

1. Lower (abaxial) leaf surfaces with scurfy, dark-centered scales; leaves gray-green; lobes of leaf blade entire . . . . *Pleopeltis*
1. Lower leaf surfaces glabrous, lacking scales; leaves green; lobes of leaf blade minutely toothed . . . . *Polypodium*

1. Pleopeltis {plee-oh-PELL-tiss} Humbolt & Bonpland *ex* Willdenow 1810 • Shielded-sorus Polypodies • [Greek, *pleos*, many, and *pelte*, shield; in reference to scales that cover young sori.]

Selected reference: Andrews, E. G. and M. D. Windham. 1993. *Pleopeltis*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 324–327.

1. ***Pleopeltis polypodioides*** (Linnaeus) Andrews & Windham [like *polypodium*] var. ***michauxiana*** (Weatherby) Andrews & Windham [A. Michaux, 1746–1802]. RESURRECTION FERN; GRAY POLYPODY. Evergreen perennial. Sporulates May - October. Epiphytic on trees, or growing on shaded rocks and logs; all highland provinces; common; [Coastal Plain]. Wetland Indicator Status, UPL. It is called resurrection fern because it appears dead during dry times but wet weather will "resurrect" it and the leaves will unfold and become green again. Scales on leaf help to conserve moisture. A total of 6 varieties of this species are known, only this taxon occurs in North America. The typical variety (var. *polypodioides*) is common in the West Indies and has scales abundant on both surfaces of the leaf. Our variety is named in honor of André Michaux , a French botanist who botanized the United States and first discovered this fern. Indians made an ointment from the leaves and rootstalk for treatment of sores and ulcers (Foster and Duke 1990). Synonyms: *Polypodium polypodioides* (Linnaeus) Hitchcock— M; *Marginaria polypodioides* (Linnaeus) Tidestrom— S; *Polypodium polypodioides* (Linnaeus) Watt— R; *Polypodium polypodioides* (Linnaeus) Watt var. *michauxianum* Weatherby— L.

2. Polypodium {polly-POH-dee-um} Linnaeus 1753 • Polypodies; Rock-cap Ferns • [Greek, *poly*, and *podion*, many feet; alluding either to the knob-like leaf scars on the rhizome or the foot-like branching of the rootstock.]

Selected references: Cranfill, R. and D. M. Britton. 1983. Typification within the *Polypodium virginianum* complex (Polypodiaceae). Taxon 32: 557–560. Haufler, C. H. and M. D. Windham. 1991. New species of North American *Cystopteris* and *Polypodium*, with comments on their reticulate relationships. Amer. Fern J. 81: 7–23. Haufler, C. H., M. D. Windham, and S. A. Whitmore. 1993. *Polypodium*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 315–323.

1. **Polypodium virginianum** ★ Linnaeus [Virginian]. ROCK-CAP FERN; COMMON POLYPODY; ROCK POLYPODY. Evergreen perennial. Sporulates June - November. Shaded cliffs and rocky, wooded slopes; Cumberland Plateau and upper Piedmont; infrequent. Species of Special Concern (Freeman *et al.* 1979). Wetland Indicator Status, UPL. This species has been medicinally used for various ailments, especially coughs and other respiratory problems (Dunbar 1989). Ashes from this fern contain large amounts of potash and were used in the manufacture of glass (Abbe 1981). *Polypodium virginianum* is identified as a tetraploid. A diploid cytotype of this species is recognized by some authors as a separate species, known as *Polypodium appalachianum* Haufler & Windham (Appalachian Polypody). These two taxon are difficult to separate and do not appear to be morphologically distinct. The scales on the rhizome of *P. appalachianum* are often uniformly golden brown (sometimes weakly

bicolored), the leaves are often larger and widest near the base (but not always), the spores are less than 52μm, and there are usually more than 40 sporangiasters per sorus. *P. virginianum* usually has bicolored scales with a dark central stripe, the leaves tend to be widest near the middle, the spores are more than 52μm, and there are less than 40 sporangiasters per sorus. Both species occur in our area, *P. appalachianum* is known from Pisgah Gorge. Synonym: *Polypodium vulgare* Linnaeus— M.

11. Pteridaceae (Maidenhair Fern Family)

Selected Reference: Windham, M. D. 1993. Pteridaceae. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 122-124.

1. Leaflets fan-shaped and oblong; sori arrangement discontinuous (with obvious separation between sori) on reflexed marginal lobes of leaflets; veins of leaflets distinct . . . . *Adiantum*
1. Leaflets not fan-shaped; sori arrangement continuous (without obvious separation) along revolute (under-rolled) margins of leaflets; veins of leaflets obscure.
    2. Blades 1-pinnate to pinnate-pinnatifid; underside (abaxial surface) of leaf covered with fringed or stellate scales . . . . *Astrolepis*
    2. Blades 2- to 5-pinnate (at least proximally); underside of leaf usually without fringed or stellate scales.
        3. Ultimate segments (smallest leaf divisions) 1-4 mm long . . . . . . *Cheilanthes*
        3. Ultimate segments 8+ mm long
            4. Rachis straw-colored to greenish; leaves palmately compound or pinnatifid (both leaf types often present on same plant); terminal leaflets from 4-13 cm long . . . . *Pteris*
            4. Rachis deep brown to black; leaves pinnate to bipinnate; terminal leaflets usually less than 4 cm long . . . . *Pellaea*

1. Adiantum* {ad-ee-AN-tum} Linnaeus 1753 • Maidenhair Ferns • [Greek *adiantos*, unwetted, for the glabrous leaves, which shed raindrops.] The name "maiden-hair" is said to be an allusion to the slender black stalks of this fern (Clute 1938).

*Contributed in part by Steven J. Threlkeld

Selected references: Fernald, M. L. 1950. *Adiantum capillus-veneris* in the United States. Rhodora 52: 201-208. Paris, C. A. *Adiantum.* In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 125-130. Paris, C. A. and M. D. Windham. 1988. A Biosystematic Investigation of the *Adiantum pedatum* complex in eastern North America. Syst. Bot. 13: 240-255.

1. Fronds broader than long, fan-like; main rachis forked at the tip; leaflets borne on one side of the forked rachises . . . . *A. pedatum*
1. Fronds longer than broad, spade-like; main rachis continuous; leaflets borne on both sides of the rachis . . . . *A. capillus-veneris*

1. *Adiantum capillus-veneris* Linnaeus [Venus' hair]. VENUS'-HAIR FERN; SOUTHERN MAIDENHAIR FERN. Deciduous perennial. Sporulates June - July. Moist ledges, under cut banks, and bluffs along creeks and rivers; Cumberland Plateau, Ridge and Valley, Piedmont Plateau; infrequent; [chiefly Coastal Plain]. Wetland Indicator Status, FACU. The Latin translation of *capillus-veneris* means "Venus' hair," alluding to the fine strands of hair of the goddess of love. Medicinally this fern was used as a diuretic and to cure headaches and colds (Dunbar 1989). This fern is commonly used in the nursery trade.
2. *Adiantum pedatum* Linnaeus [palmately forking]. NORTHERN or COMMON MAIDENHAIR FERN. Deciduous perennial. Sporulates June - August. Rich woods and mesic slopes; all highland provinces; frequent; [Coastal Plain]. Wetland Indicator Status, FACU. The fern was made into a tea and used for a cure-all (Clute 1938).

2. ASTROLEPIS {ass-stroh-LEE-puss} D.M. Benham & Windham 1992 • Star-scaled Cloak Ferns • [Greek *astro*, star, and *lepis*, scale, referring to the star-like scales on the top surface of leaf blade; Asteria was the Greek goddess of the stars.]

Selected reference: Benham, D. M. and M. D. Windham. 1993. *Astrolepis*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 140–143.

1. *Astrolepis integerrima* ★ (Hooker) Benham & Windham [absolutely entire]. STAR-SCALED CLOAK FERN; FALSE CLOAK FERN. Deciduous perennial. Sporulates June - September. Exposed limestone glades; Ridge and Valley; very rare. State Rank, S1. Wetland Indicator Status, NI. This species is a disjunct from the southwestern United States. It is not known from the study area, but has been documented in Bibb County, Alabama on the Ketona limestone glades (Allison 1996). Synonym: *Notholaena integerrima* (Hooker) Hevly—L.

2. CHEILANTHES {key-LAN-theez; kay-LAN-theez} Swartz 1806 • Lip Ferns • [Greek *cheilos,* margin, and *anthus,* flower, referring to the marginal sporangia; hence the name "Lip" fern.]

Selected references: Correll, D. C. and M. C. Johnston. 1979. Manual of the Vascular Flora of Texas. The University of Texas at Dallas, Richardson, Texas. Windham, M. D. and E. W. Rabe. 1993. *Cheilanthes*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 152–169.

1. Leaves glabrous or essentially so; pinnules entire or lobed only at base . . . .

. . . . *C. alabamensis*

1. Leaves pubescent to densely tomentose; pinnules pinnatifid to pinnate.
    2. Leaves and rachis villous hirsute (undersurface of leaves and surface of rachis clearly visible through pubescence); pubescence of rachis with several noticeable dark bands (septa-like articulations) along their length; pinnules pinnatif . . . . *C. lanosa*
    2. Leaves and rachis densely tomentose (undersurface of leaves and surface of rachis nearly obscured); pubescence of rachis without noticeable dark bands along their length; pinnules usually pinnate . . . . *C. tomentosa*

1. ***Cheilanthes alabamensis*** ★ (Buckley) Kunze [of Alabama]. ALABAMA LIP FERN. Deciduous perennial. Sporulates March – September. Forested slopes and bluffs with exposed rock (particularly calcareous); Cumberland Plateau, Ridge and Valley; infrequent; [Coastal Plain]. State Rank, previously S3 (ANHP 1994). Wetland Indicator Status, UPL.
2. ***Cheilanthes lanosa*** (Michaux) D.C. Eaton [woolly]. HAIRY LIP FERN. Deciduous perennial. Sporulates February – November. Forested slopes and bluffs with exposed, acidic rocks, ecotones of granitic flatrock communities; all highland provinces; frequent; [Coastal Plain]. Wetland Indicator Status, UPL.
3. ***Cheilanthes tomentosa*** Link [densely woolly]. WOOLLY LIP FERN. Deciduous perennial. Sporulates February – November. Forested slopes and bluffs with exposed, acidic rocks; Ridge and Valley, Piedmont Plateau; occasional; [Coastal Plain]. Wetland Indicator Status, UPL.

3. PELLAEA {pell-LEE-uh} Link 1841 • Cliff-brake Ferns • [Greek *pellos*, dark, possibly referring to bluish gray leaves.]

Selected reference: Windham, M. D. 1993. *Pellaea*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 175–186.

> Note: *Pellaea glabella* Mettenius *ex* Kuhn, Smooth Cliff-brake, occurs in adjacent Marion County, Tennessee (Cumberland Plateau) in crevices of limestone cliffs and ledges. It is currently not known from Alabama. It has glabrous petioles and rachises and the pinnae are slightly decurrent on the rachis. *P. atropurpurea* has petioles and rachises with scattered crisp hairs and the pinnae are not decurrent on the rachis.

1. ***Pellaea atropurpurea*** (Linnaeus) Link [blackish-purple]. PURPLE CLIFF-BRAKE. Deciduous perennial. Sporulates May – September. Forested slopes and bluffs with exposed rock (particularly calcareous), open, limestone outcroppings; all highland provinces; frequent; [Coastal Plain]. Wetland Indicator Status, UPL. This species was first discovered by John Clayton on the Rappahannock River in Virginia (Snyder and Bruce 1986).

4. PTERIS {TARE-iss} Linnaeus 1753 • Brake Ferns • [Greek *pteris*, fern, derived from *pteron*, wing or feather, for the closelv snaced pinnae, which give the leaves a likeness to

feathers.]

Selected reference: Nauman, C. E. 1993. *Pteris*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 132–135.

1. ***Pteris multifida*** † Poiret *ex* Lamarck [divided frequently]. SPIDER BRAKE; HUGUENOT FERN; WALL FERN. Evergreen perennial; native to east Asia. Sporulates April – November. Rockwalls, sidewalks, and open lots; Ridge and Valley, Piedmont Plateau; infrequent; [chiefly Coastal Plain]. Wetland Indicator Status, UPL. Escaped from cultivation, sporadically naturalized in our area. One of the common names is derived from the place of its first North American discovery in Huguenot Cemetery, Charleston, South Carolina (Dunbar 1989). Synonym: *Pycnodoria multifida* (Poiret) Small— S.

12. THELYPTERIDACEAE (Marsh Fern Family)

Selected reference: Smith, A. R. 1993. Thelypteridaceae. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 206–222.

1. Blades 1-pinnate to 1-pinnate-pinnatifid; leaves narrowly to broadly lanceolate in outline . . . . *Thelypteris*
1. Blades 2-pinnatifid or 2-pinnate-pinnatifid; leaves broadly triangular in outline.
   2. Pinnae for most part connected by wings along rachis; blades 2-pinnatifid . . . . *Phegopteris*
   2. Pinnae free, rachis not winged; blades 2-pinnate-pinnatifid . . . . *Macrothelypteris*

1. MACROTHELYPTERIS {macro-thuh-LIP-ter-iss} (H. Itô) Ching 1963 • Maiden Ferns • [Greek *makros*, large, *thelys*, female, and *pteris*, fern.]

Selected reference: Leonard, S. W. 1972. The distribution of *Thelypteris torresiana* in the Southeastern United States. Amer. Fern. J. 62: 97–99.

1. *Macrothelypteris torresiana* † (Gaudichaud-Beaupré) Ching [L. de Torres]. MARIANA MAIDEN FERN; TORRES' FERN. Deciduous perennial; native to tropical and subtropical Asia and Africa. Sporulates June – October. Wet banks along railroad track rights-of-way, stream banks, and floodplains; Interior Low Plateau, Ridge and Valley, Piedmont Plateau; infrequent; [chiefly Coastal Plain]. Wetland Indicator Status, FAC. This species has escaped from cultivation and is naturalized in our area. First collection of this fern was in Seminole County, Florida in 1904. Named in honor of Louis de Torres a native of the Mariana Islands where this fern was originally described. Synonyms: *Thelypteris torresiana* (Gaudichaud) Alston— L.

2. PHEGOPTERIS {fee-GOP-ter-iss} (C. Presl) Fee 1852 • Beech Ferns • [Greek *phegos*, beech, and *pteris*, fern.]

1. *Phegopteris hexagonoptera* (Michaux) Fée [six-cornered fern]. BROAD BEECH FERN. Deciduous perennial. Sporulates June - October. Rich, mesic, forested, slopes, forested floodplains, and streambanks; all highland provinces; frequent; [Coastal Plain]. Wetland Indicator Status, FACU+. The specific epithet refers to the six-angled appearance of the rachis wing (Snyder and Bruce 1986). Synonyms: *Thelypteris hexagonoptera* (Michaux) Weatherby—R, L.

3. THELYPTERIS {thuh-LIP-ter-iss} Schmidel 1763 • Maiden Ferns • [Greek *thelys*, female, and *pteris*, fern.]

Selected references: Crawford, L. C. 1951. A new fern for the United States. Amer. Fern. J. 41: 15-20. Iwatsuki, K. 1964. An American species of *Stegnogramma*. Amer. Fern. J. 54: 141-145. Smith, A. R. 1971. The *Thelypteris normalis* complex in the southeastern United States. Amer. Fern. J. 61:21-32.

1. Sori elongate; sporangia minutely hairy; indusia absent; plant of sandstone bluffs . . . . *T. pilosa* var. *alabamensis*
1. Sori round or slightly oblong; sporangia glabrous; indusia present; plant not of sandstone bluffs (primarily of wetland habitats).
   2. Lower leaflets much reduced, noticeably smaller than medial leaflets . . . . *T. noveboracensis*
   2. Lower leaflets not reduced or only slightly smaller than medial pinnae.
      3. Basal veinlets and majority of other veinlets on undersurface of leaflets forked (some simple veinlets may be present); leaflets dissected to or nearly to midvein; margins of fertile leaflets strongly under-rolled (revolute) . . . . . . *T. palustris*
      3. Basal veinlets and majority of other veinlets on undersurface of leaflets simple and unforked (a few forked veinlets may be present); leaflets lobed but not dissected to midvein; margins of fertile leaflets not under-rolled.
         4. Veins on upper surface of leaflets with stout hairs (more than 0.3 mm long); leaflets often with yellowish, stalked glands; leaf blade lanceolate to triangular . . . . *T. kunthii*
         4. Veins on upper surface of leaflets glabrous, or rarely with a few scattered hairs (less than 0.2 mm long); leaflets lacking glands; leaf blade ovate-lanceolate to lanceolate-oblong . . . . *T. ovata*

1. *Thelypteris kunthii* (Desvaux) C. V. Morton [C. S. Kunth, 1788-1850]. SOUTHERN SHIELD FERN; WIDESPREAD MAIDEN FERN. Deciduous perennial. Sporulates late May - October. Creek banks and forested floodplains; Ridge and Valley; rare; [chiefly Coastal Plain]. Wetland Indicator Status, FACW. The specific epithet is in honor of Carl Sigismund Kunth, a professor of botany at Berlin (Thieret 1980). Synonyms: *Thelypteris normalis* (C.

Christensen) Moxley, *Thelypteris unca* R. St.John, *Thelypteris saxatilis* R. St.John— S.

2. *Thelypteris noveboracensis* (Linnaeus) Nieuwland [of New York]. NEW YORK FERN. Deciduous perennial. Sporulates late May - October. Rich, mesic, forested slopes, forested floodplains, pond margins, and streambanks; all highland provinces; frequent; [Coastal Plain]. Wetland Indicator Status, FAC+. The common name was given because Linnaeus reportedly received a specimen from New York (Dunbar 1989). This species is easily cultivated, but it should be noted that maiden ferns may take over a garden. Synonym: *Dryopteris noveboracensis* (Linnaeus) Gray— M.

3. *Thelypteris ovata* ★ R. P. St. John *ex* Small [ovate]. OVATE MAIDEN FERN. Evergreen perennial. Sporulates June - October. Damp, wooded limestone ledges and bluffs; Ridge and Valley; very rare; [chiefly Coastal Plain]. State Rank, S3. Wetland Indicator Status, UPL. Though not from the known project area, this species is reported to occur in Bibb County along the Little Cahaba River (Allison 1996).

4. *Thelypteris palustris* Schott [marshy] var. ***pubescens*** (Lawson) Fernald [hairy]. MARSH FERN. Deciduous perennial. Sporulates June - October. Forested floodplains and wet, open ditches, Cumberland Plateau, Ridge and Valley; infrequent; [Coastal Plain]. Wetland Indicator Status, FACW+. Both the specific epithet and common name of this species are in reference to the wet habitats in which this plant is usually found. *Thelypteris palustris* var. *palustris* is found in Eurasia. Synonyms: *Dryopteris thelypteris* (Linnaeus) Gray— M; *Thelypteris thelypteris*— S.

5. *Thelypteris pilosa* ★ (M. Martens & Galeotti) Crawford [long, soft hairs] var. ***alabamensis*** Crawford [of Alabama]. ALABAMA STREAK-SORUS FERN. Evergreen perennial. Sporulates year-round. Sandstone bluffs and overhangs in river gorges; Cumberland Plateau; very rare. Federal Status, Threatened; State Rank, S1. The vernacular name is referring to its elongated sori (most species in the genus *Thelypteris* have round sori). This species is currently not known from northeast Alabama; however, it is known from Winston County in northwest Alabama. The type colony was discovered in 1950, but was destroyed by road bridge construction (Wherry 1972). The Alabama population is disjunct from other populations of *Thelypteris pilosa* var. *alabamensis* in north Mexico, which were discovered later. The type species, *Thelypteris pilosa* var. *pilosa* ranges from southern Mexico to Central America and is larger in size. Recent research suggests that the Alabama streak-sorus fern represents a species distinct from *T. pilosa* (Wagner 1999). Synonyms: *Leptogramma pilosa* (Martens & Galeotti) L. Underwood var. *alabamensis* (Crawford) Wherry; *Stegnogramma pilosa* (Martens & Galeotti) Iwatsuki var. *alabamensis* (Crawford) Iwatsuki.

13. VITTARIACEAE (Shoestring Fern Family)

1. VITTARIA {vye-TARE-ee-uh} Smith 1793 • Shoestring Ferns • [Latin *vitta*, ribbon or stripe; referring to the linear, string-like leaves of the sporophyte.]

Selected references: Farrar, D. R. 1974. Gemmiferous fern gametophytes—Vittariaceae. Amer. J. Bot. 62: 146-155. Farrar, D. R. 1978. Problems in the identity and origin of the

Appalachian *Vittaria* gametophyte, a sporophyteless fern of the eastern United States. Amer. J. Bot 65: 1–12. Farrar, D. R. 1993. Vittariaceae. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 3+ vols. New York and Oxford. Vol. 2, pp. 187–189. Farrar, D. R. and J. T. Mickel. 1991. *Vittaria appalachiana*: A name for the "Appalachian gametophyte." Amer. Fern J. 81: 69–75.

1. ***Vittaria appalachiana*** Farrar & Mickel [Appalachian]. APPALACHIAN SHOESTRING FERN. Persistent gametophyte (sporophyte absent or abortive). Reproduces vegetatively by gemmae. Dark, moist cavities and crevices of sandstone bluffs and grottoes; Cumberland Plateau; infrequent. Wetland Indicator Status, NI. Dense colonies of this gametophyte often coat deeply shaded rock surfaces and resemble pale-green liverworts.

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The authors thank Robert Kral, Jack Short, and the late Warren Herb Wagner, Jr. for their suggestions and comments which greatly improved the manuscript. We also appreciate the grammatical review by Verna Gates and the contributions of Terri Ballard, Tim Hofmann, and Steve Threlkeld. Most importantly, this flora would have not have been possible without the fieldwork of many botanists who have collected plants in northern Alabama. Our work is founded on the collection data of their herbarium specimens. We are indebted to the authors of the following Alabama vascular floras: Blount County (Brian R. Keener 1999), Cheaha State Park (Melanie G. Bussey 1983), Cumberland Plateau (R. David Whetstone 1981), Distribution of Alabama Pteridophytes (Jack Short 1978), Dugger Mountain (Francine N. Hutchinson 1998), Etowah County (Lesley M. Hodge-Spaulding 1997), Horseblock Mountain (Susan E. Hruska 1997), Jackson County (Kristin R. Brodeur 1999), Jefferson County (J. Patrick Barber 1986), Lake Guntersville State Park (Daniel D. Spaulding 1995), Limestone County (Timothy L. Hofmann 2000), Little River Canyon (Catherine C. Dickson 1992), Madison County (Steve J. Threlkeld 1998), Randolph County (Christopher F. Nixon 1989), St. Clair County (Hayes A. Jackson 2000), and Talladega Ranger District of the Talladega National Forest (J. Mark Ballard 1995). Other individuals making significant contributions to the Jacksonvile State University herbarium are Erik Alford, Tim Atkinson, Jerry Clonts, Terri Dobson [Ballard], Robert Kral, Ken Landers, Carol Lawler, Keener Morrow, Gary Wayner, and Loretta Weninegar.

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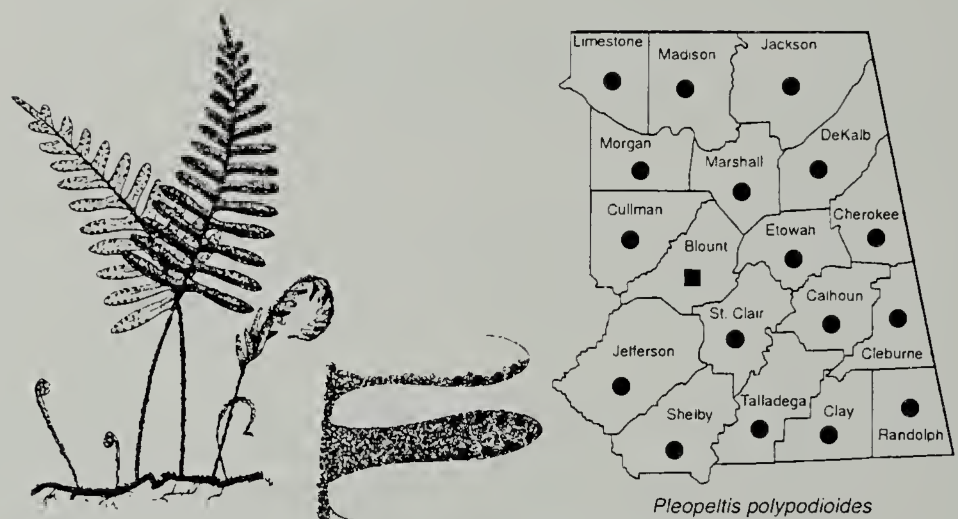


Figure 1. *Pleopeltis polypodioides*- Resurrection Fern

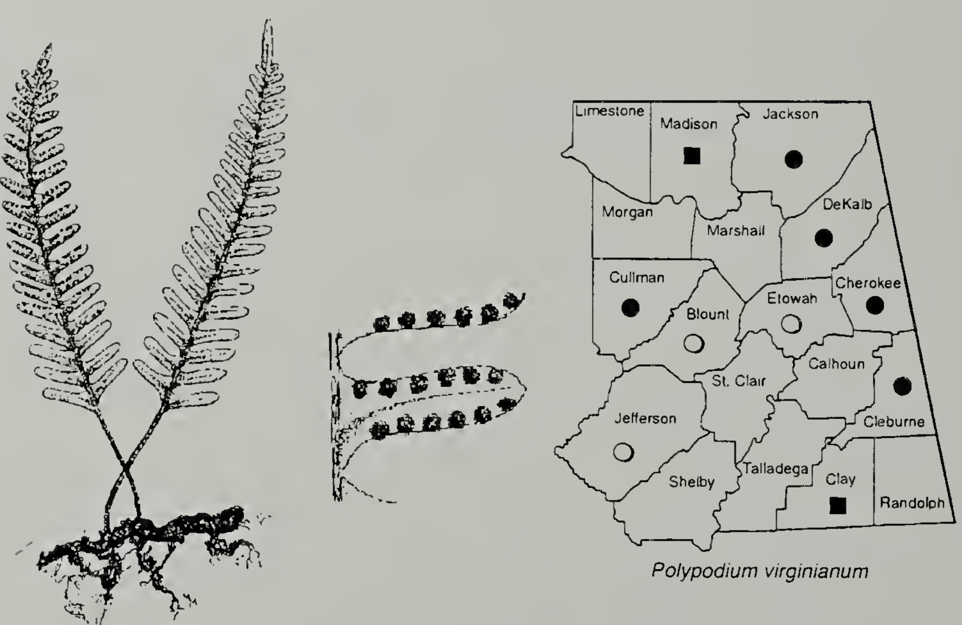


Figure 2. *Polypodium virginianum*- Rock-cap Fern

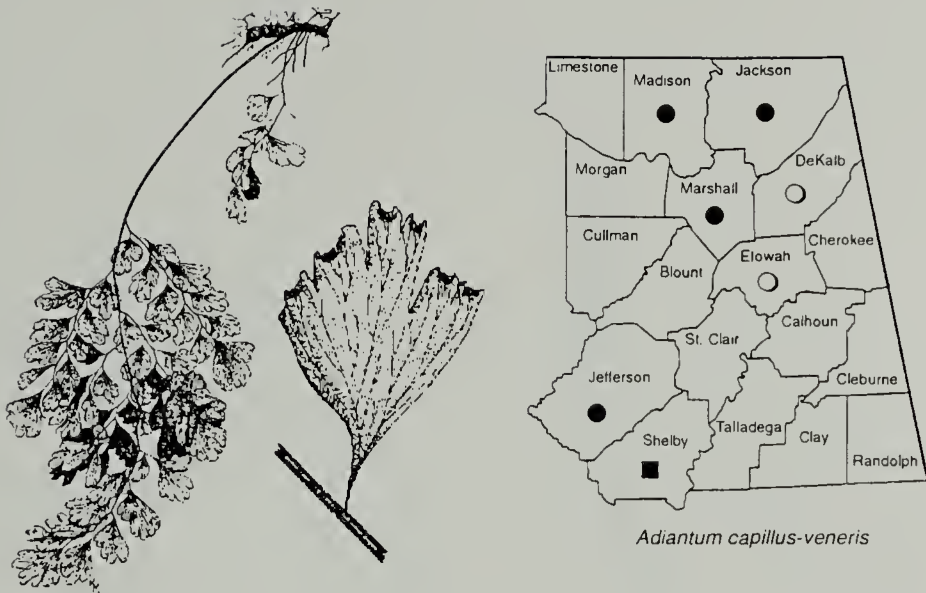


Figure 3. *Adiantum capillus-veneris*- Venus'-hair Fern

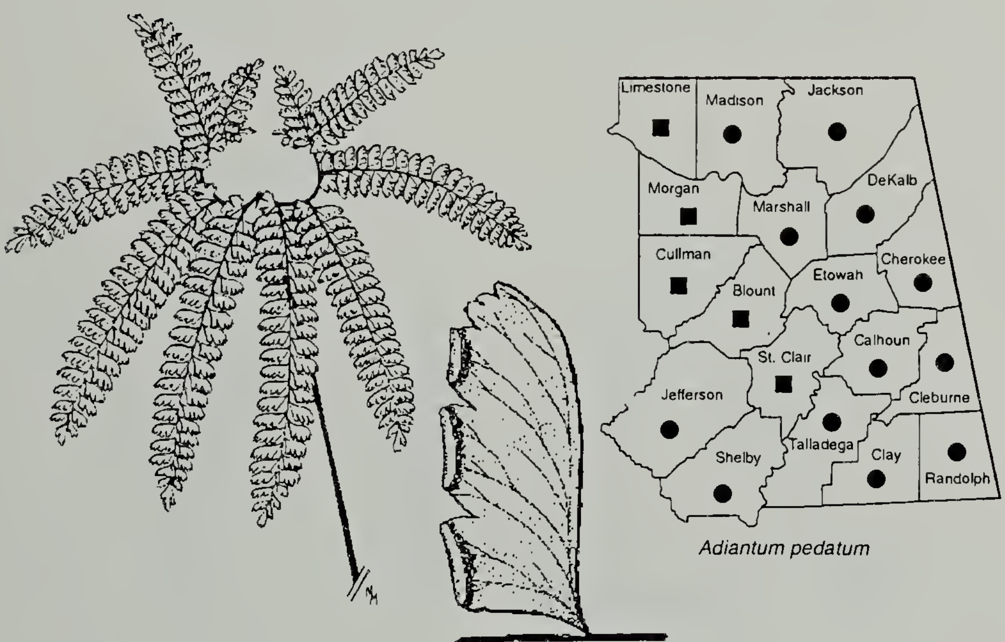


Figure 4. *Adiantum pedatum*- Northern Maiden-hair Fern

Figure 5. *Astrolepis integerrima*- Star-scaled Cloak Fern

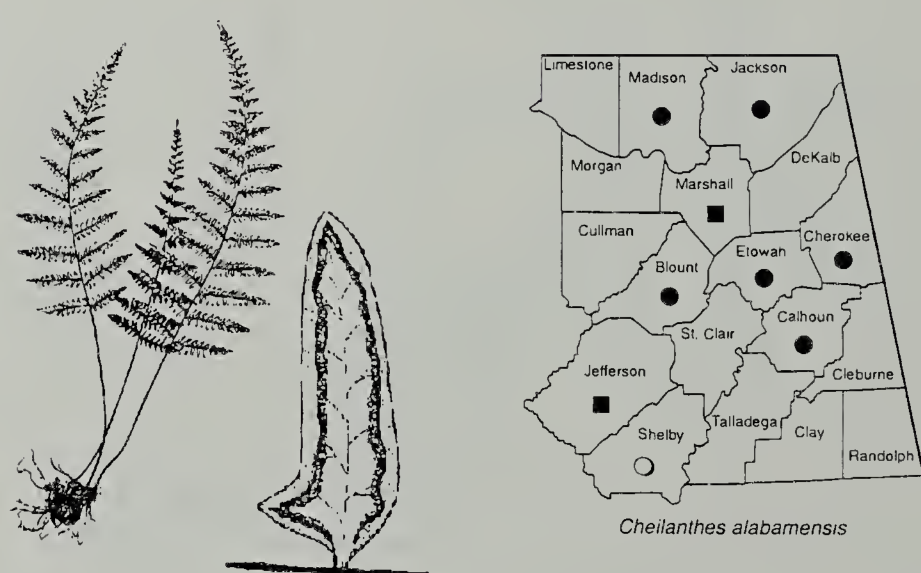


Figure 6. *Cheilanthes alabamensis*- Alabama Lip Fern

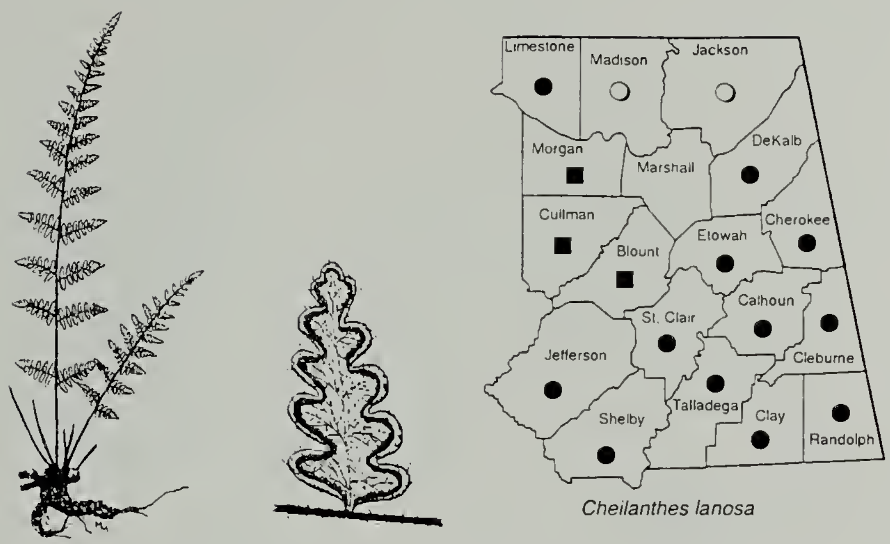


Figure 7. *Cheilanthes lanosa*- Hairy Lip Fern

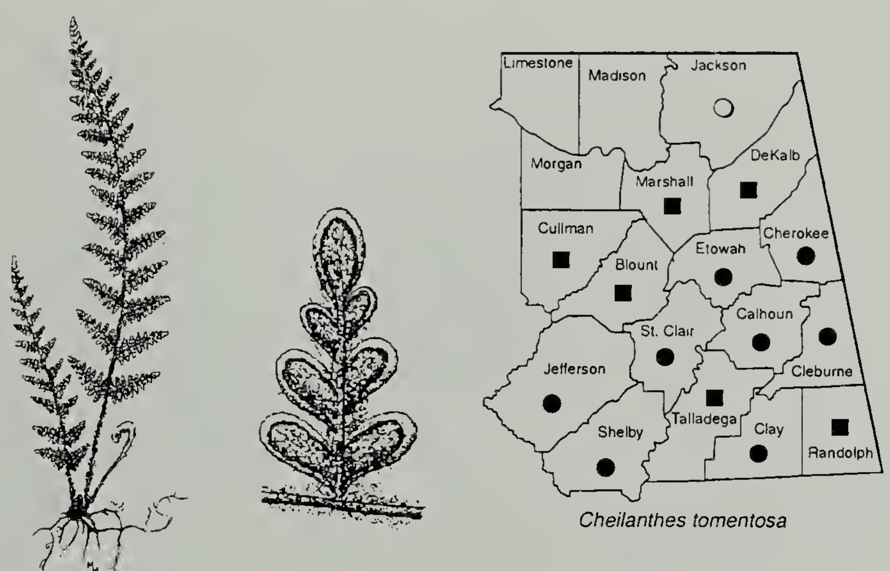


Figure 8. *Cheilanthes tomentosa*- Wolly Lip Fern

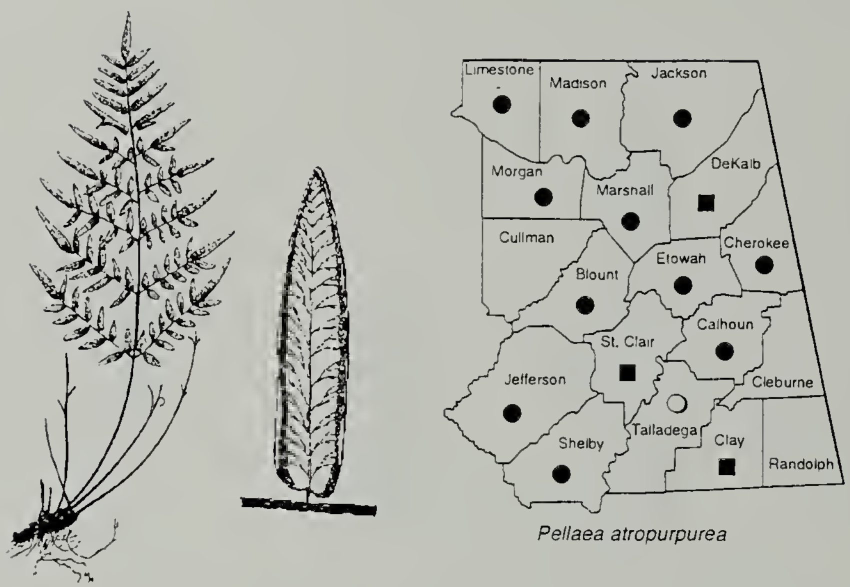


Figure 9. *Pellaea atropurpurea*- Purple Cliff-brake

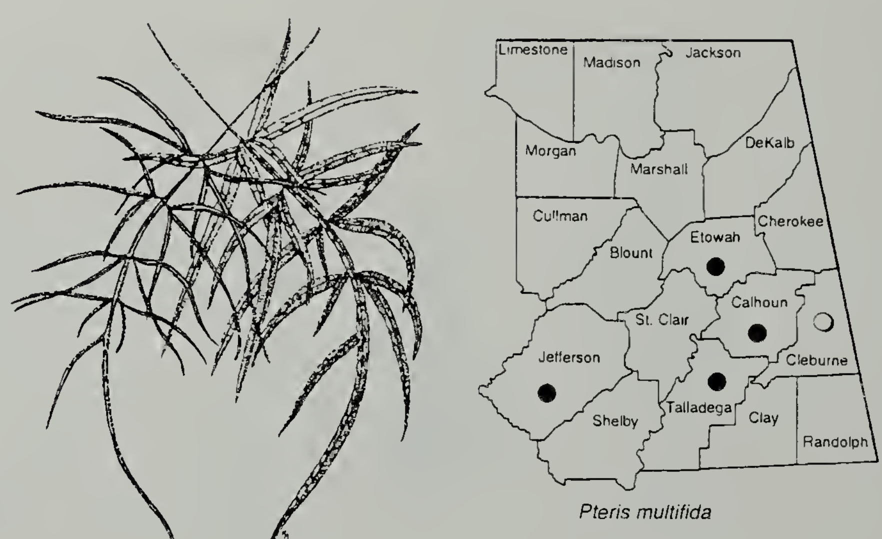
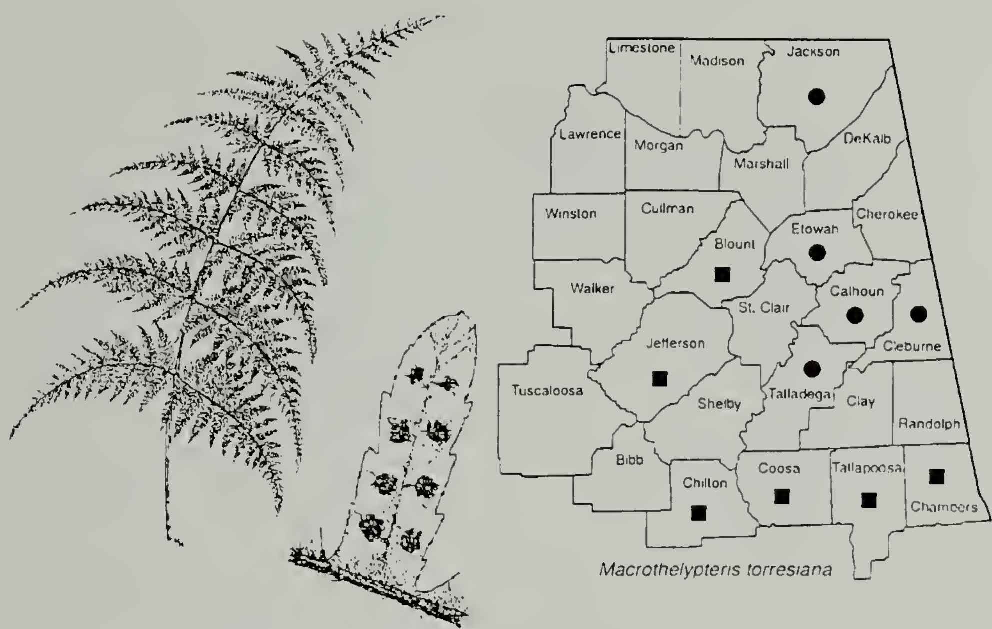


Figure 10. *Pteris multifida*- Spider Brake



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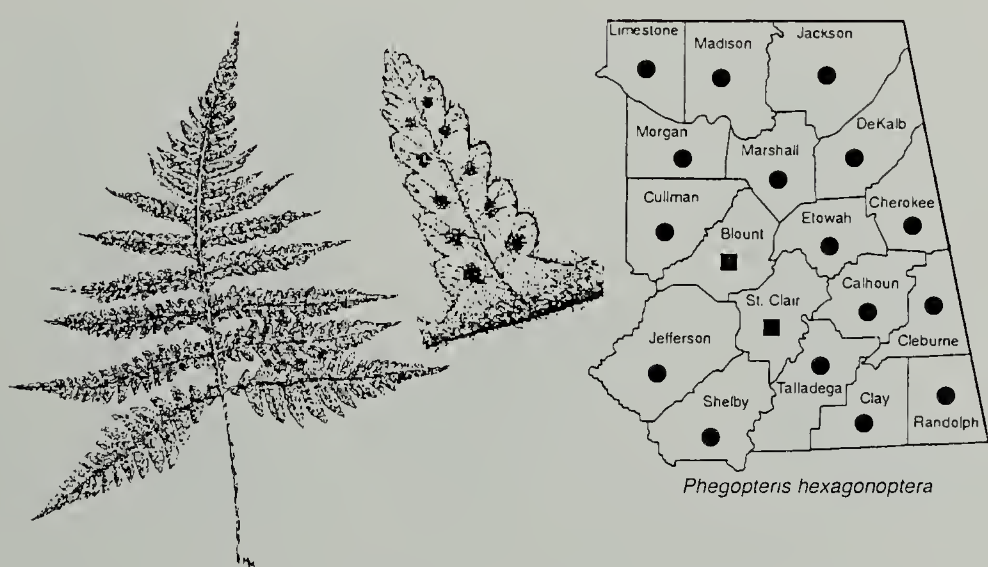

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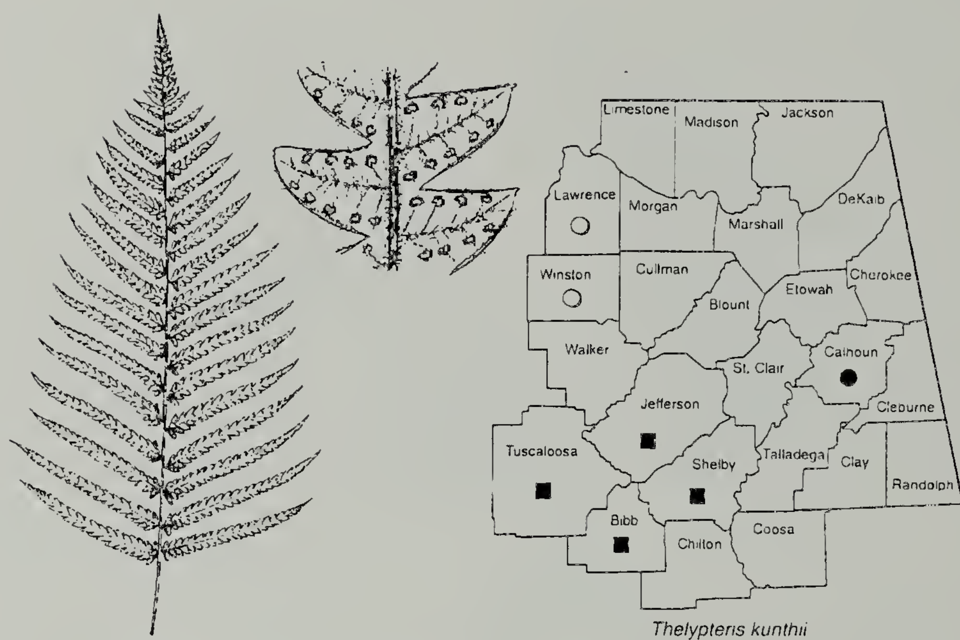
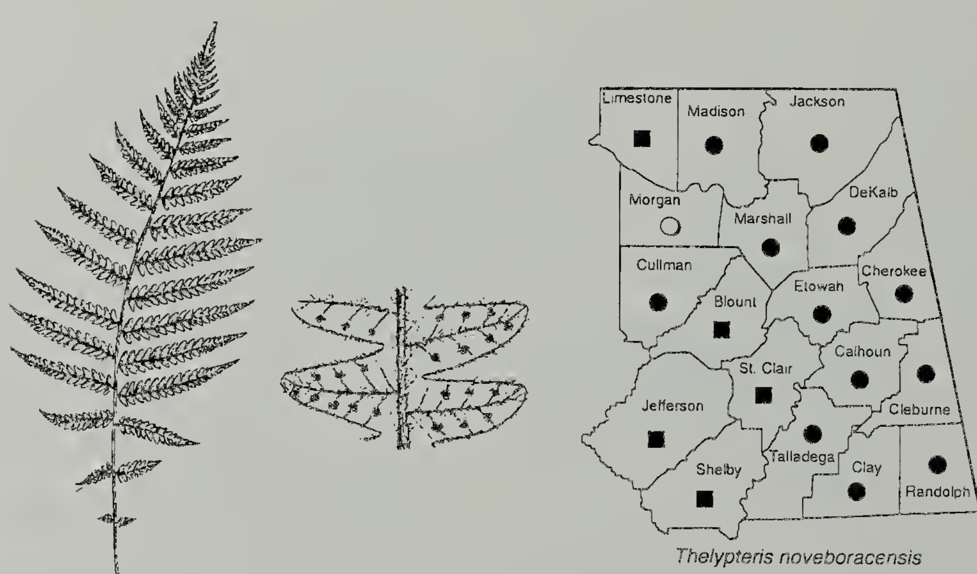


Figure 13. *Thelypteris kunthii- Southern Shield Fern*



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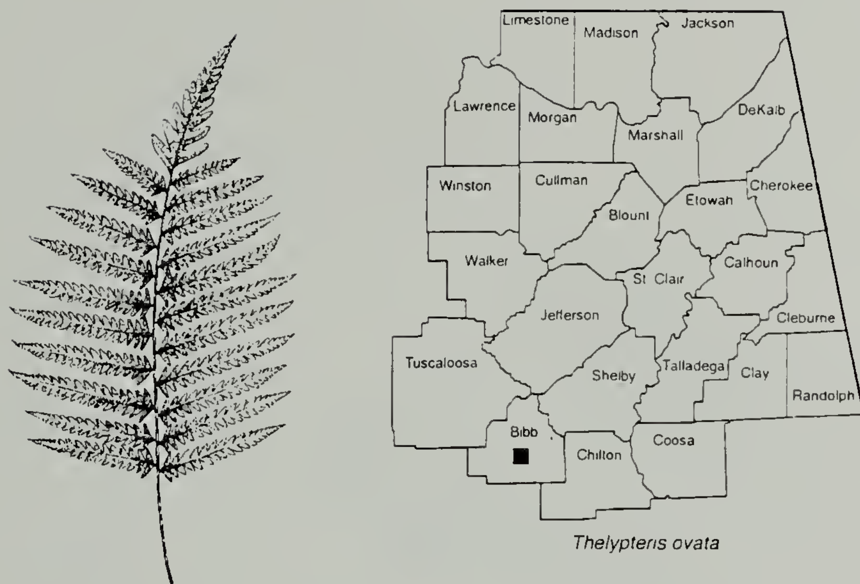

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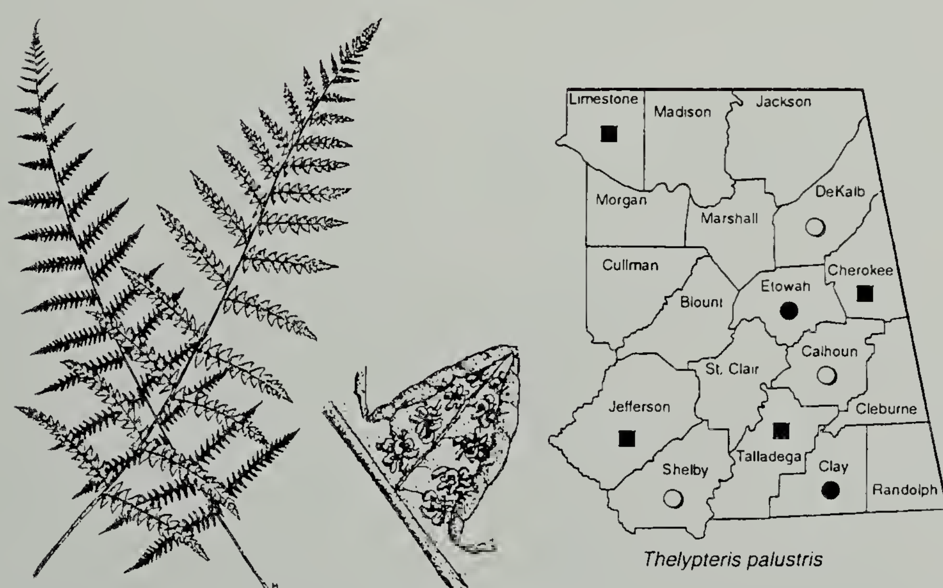

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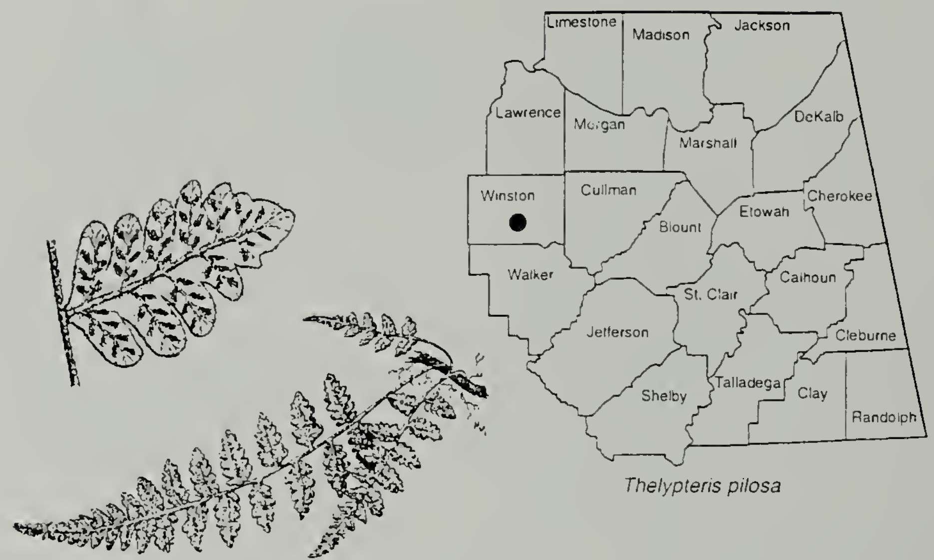
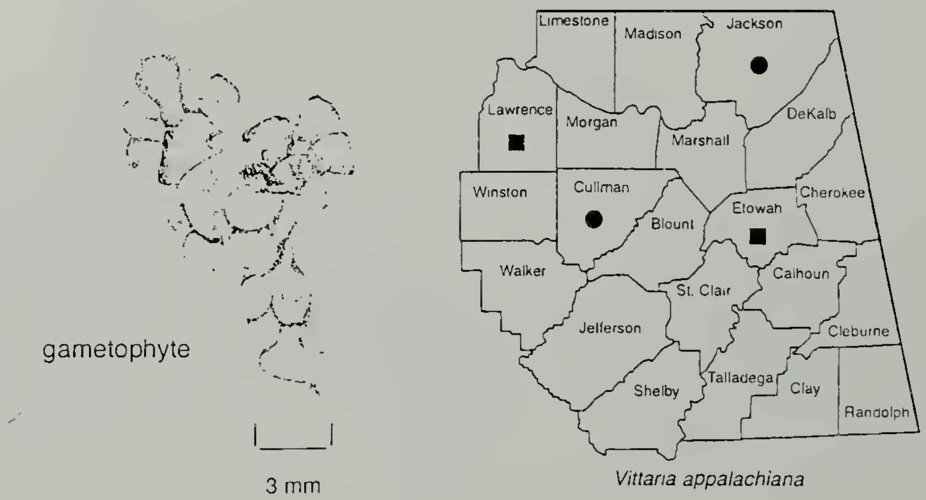


Figure 17. *Thelypteris pilosa*- Alabama Streak-sorus Fern



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BOOK REVIEW

HUMAN EVOLUTION IN SEARCH OF AN EXPLANATION

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The Riddled Chain: Chance, Coincidence, and Chaos in Human Evolution, Jeffrey K. McKee. x + 280 pp. Brunswick: Rutgers University Press, 2000. $27.00.

About 5 million years ago a divergence in primate evolution gave rise to the great ape lineage on the one hand and hominids including modern humans on the other. The fossilized common ancestor to these two lineages has yet to be found. We know that by at least 4 million years ago animals in the hominid lineage had taken to walking upright. At about 2.5 million years ago the hominid lineage diverged to produce robust, thick-jawed vegetable-eating animals and a line of more gracile hominids which included meat in their diet and made stone tools. The meat-eating toolmakers ultimately gave rise to extant tofu-eating internet users; whereas, the robust vegetarians became extinct.

Why, asks McKee, did early hominids become bipedal in the first place? And why did our gracile ancestors survive while the robust branch of the hominid line disappeared? Mckee's answer to these "why" questions is that there is no answer at all. He discards the view that natural selection triggered by climatic changes is an adequate explanation for human evolution. Instead, he maintains that the major events in human evolution were triggered by chance, coincidence and chaos.

If chance and other vagaries resulted in bipedalism, then our large brains, manual dexterity, and ability for spoken language are still in need of an explanation. In fact, McKee writes, "...is there really more to human evolution than our theories have captured so far? Despite our faults, humans are in need of an explanation...The explanation is *autocatalysis* (my italics)."

Descriptions, explanations, and examples of what is meant by chance, chaos, coincidence, and autocatalysis in human evolution occupy the major part of the book, and these all deserve comment and some criticism. But first it seems right to state that this is not just a book about human evolution. It is also a highly personal account of the scientific process. McKee is a practiced physical anthropologist/theoretical evolutionary biologist now at The Ohio State University who states at the outset that he believes science is fun. He communicates this enthusiastically and contagiously on virtually every page using down-to-earth language and engaging stories. His personal accounts of what life is like for a field anthropologist camping out in South Africa make science fun for the reader as well.

That science is not a collection of observations or "facts" but rather a non-dogmatic, lf-correcting process by which we endeavor to understand the world and our place in it is

also communicated very well in this book. The story of human evolution as a scientific discipline is littered with discarded and disproven hypotheses. McKee describes several of these and how each was an exciting and promising explanation for the data on hand at the time of its formulation. How new data produces new hypotheses and ultimately theories is clearly shown by McKee's examples. The relevance of these anthropological examples to other domains of science should be easy to recognize by most readers. Thus, non-scientists who often become frustrated with the apparent qualified or tentative nature of "scientific facts" - eggs are bad for you, no they are good; alcohol is bad for you, except in red wine; breast self-examinations save lives, no they don't; supplementary vitamin C prevents cancer, no sorry it causes it - should gain some tolerance and understanding for the changing views of science by reading this book.

Now, back to chance, coincidence and chaos, "three ubiquitous and mischievous forces" claimed by McKee to be major players in producing all life forms on Earth. Three to 7 pages in Chapter 1 are devoted to describing each "force."

Chance is what makes the existence of every living individual highly unlikely. McKee tells us that the chance of his mother and father being born female and male and of himself being born the last of four sons had less than a 1.6% probability. Multiply this by the likelihood that a particular egg and sperm would have united to produce the zygote that became Dr. McKee and you begin to get his drift.

Coincidence acquires an ambiguous meaning in McKee's hands. At first it appears to come in two varieties, that due to common cause and that not explicable by a common cause: "Coincidence, be it by cause or caprice, is part of all lives" (p. 9). But later on the same page, in a discussion of cause and effect in evolution, it is stated that "discerning the difference between mere coincidence and important causation is not only difficult but is severely hampered by the fragmentary nature of the fossil record." For example, McKee shows in Chapter 4 that peaks of first appearance of certain mammals in the fossil record need not have been caused by coincident changes in climate, but could be due simply to chances of fossilization and discovery. Although *coincidence* is never defined in the book, I will call it *the occurring of two or more events at virtually the same time or place when no apparent cause for such occurrence can be discerned.* This is consistent with dictionary definitions, and also I believe it is fair to the author's most frequent use of the term.

Chaos is defined. It "represents unpredictability based on sensitivity to initial conditions" (p. 13). Examples cited include versions of the famous "butterfly effect" (p. 14) whereby the beating of a butterfly's wings in Florence, Italy, may result in a hurricane making landfall in New Orleans six weeks later. The extreme sensitivity of chaotic systems to initial conditions makes them unpredictable and thereby results in the *appearance* of chance. Not only weather, but also evolution, is subject to chaos theory according to McKee: "Chaos develops when the various forces of evolution combine" (p. 16). This makes it impossible for our limited analyses of past climates, geographies, ecosystem compositions, hominid morphologies, etc. to yield an airtight, causative explanation for human evolution.

The first seven chapters of the book are devoted to explaining how chance, coincidence and chaos may have been more important in human evolution than the forces of environmental change acting through natural selection. This prepares us for Chapter 8 which

presents the centerpiece of the book, *autocatalysis*.

"...autocatalytic evolution is a concept so simple, so basic, that it is actually difficult to explain," writes McKee (p. 204). But earlier it is described quite clearly: "Autocatalytic evolution simply means this: evolution is caused (catalyzed) by itself (auto). It is self-propelled by feedback loops" (p. 202).

Autocatalysis in human evolution refers to the interplay between bipedalism, brain elaboration, manual dexterity, dietary niche, language and material culture - each of these reinforcing the others through positive natural selection. It is suggested that the *chance* occurrence of bipedalism among our forest-dwelling primate ancestors along with the *coincidental* by-product of free hands (p. 205) set all of this into motion. (Note that this use of the word "coincidental" includes causation and therefore does not jibe with earlier descriptions of the term.) Later the *adaptability* allowed by an expanded neocortex in our larger brained, gracile hominid ancestors proved more successful than the *adaptation* of a massive skull and jaw for chewing vegetables acquired by the robust lineage of hominids.

These ideas are not new. McKee acknowledges that even Charles Darwin and Thomas Huxley had thought of the positive reinforcement that bipedalism, brain size, and tool use might have on each other. Edward O. Wilson discusses autocatalysis extensively in his book *Sociobiology* (Belknap Press of Harvard University Press, Cambridge, MA, 1977), although the term is absent from the glossary and index of the 2nd edition of Mark Ridley's widely used text, *Evolution* (Blackwell Science, Inc., Cambridge, MA).

McKee claims that autocatalytic evolution "holds a lot of explanatory power lacking from the neo-Darwinian synthesis of evolutionary theory (p. 202)...and that the theory behind it should be more thoroughly investigated, for a lot of mammalian evolution, perhaps most of evolution, may be a product of autocatalysis" (p. 203). No examples of possible autocatalysis playing a role in the evolution of non-human organisms are given, although this is forgivable since the book is about human evolution. One would hope that data or a book would be forthcoming though to back up the suggestion of such great importance for autocatalysis in all of evolution.

The emphasis on the three "c" words seems a bit concocted to me. From what is written, *chance* is only an illusion created by *chaos*, and the unpredictability of chaos is simply due to our incomplete knowledge about initial conditions. From this doesn't it follow that nothing is really *coincidental* at all, at least in the sense of lacking causal connections to everything else? Although I suspect that the book is less ground-breaking than some of its passages imply, it is a "good read" for biologists and non-biologists alike. Non-scientists will gain an appreciation for the process of science, and every reader will have her imagination and interest tweaked by the discussions of how we came to be human.

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Donaldson, Steve
Drake, John M.
Feinstein, David L.
Francis, Lara
Garza, Gene G.
Hearn, William H.
Hilyer, William A.
Hu, Bei
Jacobs, Paul L.
Karam, Marc
Kurzius, Shelby C.
Lee, Beum-Seuk
Lokey, Laura
Lu, Yashui
Lumpkin, Sam
Panwhar, Samina
Park, Jung-me
Parker, Donald L.
Pitt, Robert E.
Pun, Oceana
Raje, Rajeev R.
Raju, P.K.
Ray, Cari
Ren, Jing
Roy, Sanjeev R.
Selvaraj, Madhanraj
Sloan, Kenneth R.
Sprague, Alan P.
Srinivas, Raghavan N.
Tao, Tao
Thomas, Robert E.
Walters, J.V.
Wang, Xin
Wang, Yibing
Wisniewski, Raymond B.
Wong, Daisy
Wu, Xiaqing
Yang, Chunmin
Yerramsetti, Ramesh
York, Gary
Zhang, Mila
Zheng, Xidong
Zhou, Song

SECTION XI
ANTHROPOLOGY
Driskell, Boyce N.
Gage, Matthew D.
Henson, B. Bart
Holstein, Harry O.
Hurley, Molly
Mann, Jason A.
Rowe, Bobby
Runquist, Jeanette
Shelby, Thomas M.
Speegle, Heath F.
Twe, Kyla Elizabeth

Minutes
AAS Fall Executive Committee Meeting
Southern Research Institute Library
Birmingham, Alabama
October 20, 2001

Call to Order and Approval of Minutes (A) After a brief moment of silence on behalf of American military forces engaged in hostilities abroad, President Roland Dute called the meeting to order at 10:16am. The minutes of the Spring meeting of the Executive Committee (March 28, 2001) were approved.

Officer Reports (B)

1. Eugene Omasta (**Board of Trustees**) had no written report, and indicated that he would report later for the **Finance Committee**.

2. Roland Dute (**President**) reported the following activities as part of the duties of the office:
   - Helped Stephen Watts (**First Vice-President**) locate people for the appointed committees;
   - Contacted Amy Sheldon (Alabama Imaging and Microscopy Society) about the possibility of a joint meeting;
   - Set a date for the Fall meeting;
   - Attended site visit at the University of West Alabama;
   - Helped coordinate transfer of information regarding the annual meeting from the Local Arrangements Committee at Auburn University to a similar committee at the University of West Alabama;
   - Kept the Executive Director informed of any and all changes;
   - Engaged in constant interactions with officers and members;
   - Represented the Academy at Dr. J. H. M. Henderson's retirement dinner at Tuskegee University

3. Stephen Watts (**First Vice-President**) reported the following activities:
   - Worked with Dr. Dute to fill some of the vacancies involved in several of the committees;
   - Continued to press for the continued development of AAS electronic databases and web pages as a means of mitigating some of the complexities of the Society;
   - Participated in the site visit to the University of West Alabama;
   - Worked together with Anne Cusic, Roland Dute and Dail Mullins to redesign the dues cards to allow for a multiple-year option;
   - Had several contacts with the American Association for the Advancement of Science in my role as representative to that organization;
   - Reported that he would like to revisit the option of charging a $5-10 abstract fee to help raise money for the Journal.

4. Anne Cusic (**Second Vice-President**) reported that she has spent some time trying to figure out why she agreed to take on the task of Second Vice-President. In addition, she has been talking to Dr. Watts and Dr. Dute about her duties and how she can help move the Academy along. She indicated that she has also participated in discussions about the student research awards.

5. The **Secretary** (Dail Mullins) submitted a written report in which he indicated the following activities: (a) transferred all checks/cash received for dues to the Treasurer after recording information on the master roll; (b) supplied the editor of the *JAAS* with membership rolls and mailing labels as requested; (c) made all requested mailing address changes to the master roll; (d) forwarded several requests for reprinted articles from the *JAAS* to the editor; (e) sent membership lists to the Section Heads as requested by the Executive Director; (f) redesigned the dues cards to reflect the option of paying for 1, 2, or 3 years as requested by the Executive Director; (g) submitted minutes of the spring AAS Executive Committee meeting to the editor of the *JAAS*; and (h) submitted the names of two high school students (Mr. Jordan Farina, Killen, AL, and Ms. Mary Cole, Demopolis, AL) and two college students (Ms. Susan Green, UAB, and Mr. Jason Stanko, UAB) as nominees for a complimentary membership in AAAS.

6. Larry Krannich (**Treasurer**) submitted a lengthy written report which included: (a) a statement of all Account Balances as of Oct. 12, 2001; (b) an Income and Expense statement as of Oct. 12, 2001; (c) Activities Relative to the 2000 Budget for the period 1/1/01 through 12/12/01; (d) a Treasurer's Summary Report by Quarter for the period 1/1/01 through 10/12/01; (e) a Treasurer's Summary Report by Account for the period 1/1/01 through 10/12/01; and (f) a Proposed Budget 2002 vs 2001.

   The total funds in all accounts has decreased by $1,051.09 since the 2000 Fall Treasurer's Report. This includes the revenue from two Annual Meetings that were held on the Samford University campus in 2000 and Auburn University campus in 2001. Dues revenue for the first nine months in 2001 is almost twice that for the same time-frame in 2000, but this is a result of almost no dues being collected in the fourth quarter of 2000. In terms of a two year period, we have collected on $11,021 in dues while we anticipated collecting $18,000. Thus, we have a negative variance from projections of approximately $7,000 over a two year period, or $3,500 per year. Support for the journal was just slightly above budget again this year. In the Gorgas, Science Olympiad, and Science Fair categories, we traditionally receive funds which offset the expenses in these categories. This year, all Science Fair finances were handled directly by the Academy. Science Fair funds ($21,631) were transmitted to the Academy and we paid all expenses for student participation in the International Science Fair. On the expense side, we can anticipate to pay approximately $6,500 for the publishing of two more journal editions in 2000, because all editorial work has been completed on these. The bond on the Treasurer expenses represents a three year premium, while the budget amount is for annual cost. In general, we are within the budge that was adopted for 2001. The Proposed Budget for 2002 is identical to the actual 2001 budget, except for the State Science Fair category.

7. The **Editor of the *JAAS*** (Jim Bradley) submitted a written report. The Journal seems to be thriving with increased numbers of submissions and continued support from the Auburn University Library for the current fiscal year (ends 9/30/02). The AU Library no longer processes the Journal for mailing. Sue Bradley has agreed to perform this service at an hourly rate beginning with the April issue which has just been delivered from the printer. Thus far, three of the four 2001 issues of the Journal are published. The abstract issue (April) is being mailed out next week, and the July issue is printed but not yet delivered for mailing. The April, 2001 issue contains 118 abstracts from the March meeting, down somewhat from the 133 abstracts published from the 2000 meeting. The July, 2001 issue is a special issue containing five of the six Bioethics Symposium papers presented at the March meeting plus a book review on bioethics and the human genome. Publication of the *JAAS* is nearly back on schedule. The October, 2001 issue is expected to go to press in late November. The Editor reaffirmed his recommendation of last year that the Academy continue to publish a hardcopy journal with four issues per year. A $5.00 increase in membership dues would more than make up for an anticipated future loss of support from the AU Library.

8. The **Counselor to AJAS** (B. J. Bateman) submitted a written report. He indicated that the ASTA Meeting last week in Mobile was poorly attended, with rumors circulating that Birmingham area teachers had boycotted the conference. Newly elected officers for 2000-2001 include: Michael Vincint (President, JCIB); Mary Cole (Vice-President, Demopolis H.S.); Joy McCampbell (Treasurer, Demopolis H.S.); Rebekah Rogers (Secretary, Bradshaw H.S.).

9. Virginia Valardi (**Science Fair Coordinator**), who took over duties from Ms. Thomaskutty, submitted a written report. 18 Finalists, 1 student observer, 1 Science Fair Coordinator, and 21 teachers, parents or Fair Directors attended the 2001 INTEL/ISEF in San Jose, CA, in May. Ms. Valardi reported that there were no problems on the trip, and that the children were well-behaved. Finalists from Alabama won the following awards at the INTEL/ISEF Fair: <u>Special Awards</u>—American Association for Clinical Chemistry: Adam Grant Georgas, UMS Wright Preparatory School, Mobile; American Association of Physics Teachers and the American Physical Society: Nicole Anne Oertli, Murphy H.S., Mobile; United Technologies Corporation: Nicole Anne Oertli, Murphy H.S., Mobile. <u>Government Industry and College Scholarships</u>—Patent and Trademark Office/U.S. Dept. of Commerce: Nicole Anne Oertli, Murphy H.S., Mobile; California State University, Dominguez Hills: Julie June Bucy, The Altamont School, Birmingham; San Jose State University-College of Science and College of Engineering: Bryce Leitner Roberts, Mountain Brook H.S. <u>Grand Award</u>—Behavioral and Social Sciences: Anna Marie Deason, JCIBS, Birmingham; Engineering: Bryce Leitner Roberts, Mountain Brook H.S., Birmingham; Physics: Nicole Anne Oertli, Murphy H.S., Mobile. The 2002 INTEL/ISEF Fair will be held in Louisville, KY, May 12-18. All finalists will travel by bus with pick-up points in Mobile, Montgomery, Birmingham and Huntsville. The Regional Fair dates are as follows: North Alabama (Mar 11-14); West Alabama (Mar 15-16); Talladega (Feb 28-Mar 1); Birmingham (Mar 1-2); South Alabama (?). The State Science Fair will be held in Huntsville (April 14-16).

10. The **Science Olympiad Coordinator** (Jane Nall) submitted a written report. As of Friday, Oct. 19, there were 76 teams registered. Also, Dr. Nall reported no problems with the Colorado State visit. The dates for the Alabama Science Olympiad 2001-2002 are as follows:
    - **Elementary Science Olympiad Tournaments** (Geneva H.S., Nov. 3; Jacksonville H.S., Feb. 23; Auburn University, April 20)
    - **Secondary Regional Science Olympiad Tournaments** (University of Alabama, Feb. 9; Auburn University, Feb. 16; University of Alabama in Huntsville, Feb. 23; University of South Alabama, Mar. 9; Jacksonville State University, Feb. 23)
    - **Secondary State Science Olympiad Tournaments** (Huntingdon College, April 6; Troy State University, TBA)
    - **National Science Olympiad** (University of Delaware, Newark, DE, May 17-18)

11. Steven Watts (**Counselor to AAAS**) reported that he had made contact with the AAAS, and that it has been some time since such contact with the AAS had been made. The national organization will continue to correspond with Dr. Watts by email.

12. **Section Officers** – written reports were turned in for Sections V (Physics and Mathematics) and VIII (Behavioral and Social Sciences) only.
    - Section I (Biological Sciences, Donald Salter)—26 oral presentations, 1 poster
    - Section II (Chemistry, Steven Arnold)—full schedule, no competition papers
    - Section III (Geology and Earth Science, David Allison)—no report
    - Section IV (Geography, Forestry, Conservation and Planning, Chakudi Izeogu)—no report
    - Section V (Physics and Mathematics, Govind Menon)—the Section hosted a total of 14 presentations, 12 oral and 2 posters. The Section Chair intends to send out letters to all universities/colleges in the state which offer degrees in physics and mathematics, encouraging participation of both faculty and students.
    - Section VI (Industry and Economics, Eric Rahimian)—the chair reported that some faculty had complained that the *JAAS* published only abstracts and not entire articles.
    - Section VII (Science Education, Jane Nall)—the chair reported that they have had a full complement of presenters
    - Section VIII (Behavioral and Social Sciences, Janice Wittekind)—chair reported a total of 13 papers, of which 12 were presented. There was some student participation. In addition, Section VIII has established several goals for the 2001-02 academic year: (1) increase visibility of AAS; (2) increase participation from institutions throughout the state; (3) increase both faculty and student participation; (4) encourage manuscript submissions to the *JAAS*.
    - Section IX (Health Sciences, Robert Pieroni)—Ellen Buckner reported for Dr. Pieroni
    - Section X (Engineering and Computer Science, Alan Sprague)—Dr. Sprague reported that the Section has a new vice-chair, Dr. Robert Pitt.
    - Section XI (Anthropology, Harry Holstein)—no report.

13. Lev Hazelgrove (**Executive Director**) submitted a written report of activities over the past seven months: (1) with the leadership of Dr. Dute and Dr. Salter, UWA Local Arrangements Chair, set up site visit for Friday, July 13, 2001. Drs. Buckner, Bateman, Omasta, Watts, Dute and 12 UWA faculty attended; (2) with the leadership of Dr. David Nelson, USA Dept. of Biology, Dr. Tom Bilbo, University of Mobile Dept. of Biology, Dr. Jane Nall, Dr. Ellen Buckner, and Dr. Regan, set up the AAS booth at Davidson H.S. in Mobile for the ASTA (Oct. 4-6); (3) represented AAS at the Alabama Fisheries Association with Dr. Ken Marion and Dr. Robert Angus, Lake Eufala, Feb. 14-16; (4) helped to successfully send a team of 19 H.S. students to the INTEL/ISEF in San Jose, CA, May 6-12; (5) prepared the Gorgas Scholarship Report for Alabama Power Foundation meeting, Oct. 19.

<u>Committee Reports</u> (C)

1. **Local Arrangements** (Don Salter)—Dr. Salter reported that the site visit to UWA in July went well, and a good start has been made in preparing for the AAS annual meeting, March 27-30. Dr. Salter also reported that: (a) the Senior AAS will have all scientific sessions and business meetings in Bibb Graves Hall, while the JAAS and Gorgas Scholarship will have their sessions in Wallace Hall; (b) registration facilities and continental breakfasts for Senior AAS will be in Webb Hall Parlor and the adjacent Webb Hall Gallery on Wednesday—Friday, March 27-29. Registration facilities for the JAAS and Gorgas participants will take place at the Livingston Motel. Continental breakfasts for JAAS and Gorgas participants will take place on the second floor of Wallace Hall, Thursday—Saturday, March 28-30; (c) the Executive Dinner on Wednesday night, March 27, will take place in the Student Union Building (SUB) Conference Room. UWA Dining Services will cater the dinner. The cost will be approximately $10; (d) the poster and vendors' exhibit will take place nearby in Pruitt Hall (Gymnasium) on Thursday, Mar. 28 through Friday, Mar. 29. Poster boards will probably be borrowed from the Mississippi Academy of Science organization through Mr. Sammy Culpepper. Letters and emails will be sent to various vendors after Thanksgiving; (e) a catfish social on Thursday, Mar. 28 will take place in the Livingston Community Civic Center, hosted by Mr. Micky Smith, Department of Mathematics; (f) the Symposium on Friday morning, March 29, will be given in Bibb Graves Auditorium. The theme will be "Geology and Fossils of Alabama"; (g) the Annual Banquet on Friday, March 29, will take place in the Livingston Community Civic Center, and UWA Dining Services will cater the event. The cost will be approximately $12. Mr. Al Shotz, Nature Conservatory, has agreed to be the banquet speaker and will talk on the "Biodiversity of Alabama"; (h) the social event for the JAAS and Gorgas participants after the banquet will consist of games and movies in the basement of the SUB.

2. **Finance** (Eugene Omasta)—the Alabama Academy of Science continues to be in excellent financial condition with total assets of $71,763. However, problems are emerging. Even though the assets tend to vary from year to year for a variety of cash flow reasons, the assets are decreasing.

	Assets	Change
Fall 1998	**$56,935**	
Fall 1999	**76,219**	**+ $19,284**
Fall 2000	**72,814**	**- 3,405**
Fall 2001	**71,763**	**- 1,051**

The problems are apparent: (1) as reflected in the Treasurer's Report, dues income is approximately $7,000 lower than projections over the past two years; and (2) Journal costs continue to rise. The Trustees are examining these two problems. Essentially the Treasurer's proposed budget for 2002 is a repeat of the 2001 budget, and I recommend its acceptance.

3. **Membership** (Mark Meade)—no report

4. **Research** (Larry Boots)—no report

5. **Long-Range Planning** (Ken Marion)—reports that issues previously raised (see Minutes of Spring Meeting) keep "popping-up": (1) possibly fixing a central area for the annual meeting; (2) explore the possibility of joint meetings with other scientific organizations in the state; (3) web site and web site maintenance; and (4) a regularly appearing newsletter.

6. **Auditing-Senior Academy** (David Schedler)—no report

7. **Auditing-Junior Academy** (Danice Costes)—a written report, given by B. J. Bateman, was submitted. To quote the report: "We have examined the books provided by the Alabama Junior Academy of Science Treasurer, Dr. B. J. Bateman. We are satisfied ourselves that the receipts and expenditures, as presented to us, are correct and that all expenditures are legitimate expenses." The net worth as of Jun 30, 2001 is $15,604.04.

8. **Editorial Board and Associate Journal Editors** (Thane Wibbels, Larry Witt, William Osterhoff)—no report.

9. **Place and Date of Meeting** (Thomas Bilbo)—no report. Dr. Dute did indicate that the 2002 meeting will be held at the University of West Alabama, March 27-30, and the 2003 meeting at Jacksonville State University, March 19-22.

10. **Newsletter/Electronic Media** (Richard Hudiburg)—Dr. Hudiburg reported the following activities: (1) prepared an ad for the program of the 2001 Alabama Science Teachers Association annual meeting; (2) had several discussions with Dr. Dute concerning internet online submissions for student travel and paper/poster competitions; (3) updated the web pages for the Academy on the current server at the University of North Alabama (http://www2.una.edu/psychology/aas.htm), and a redirect link from the old website at Athens State University was established to the current server; (4) up-dated web application materials for the Committee on Research in preparation for the 79th annual meeting at the University of West Alabama, and developed two on-line application forms for students (travel paper/poster competitions)(http://www2.una.edu/psychology/aaspage.htm);

many web hosting companies, and prices vary from $9.95/month. Ellen Buckner moved, and it was seconded, that we accept this recommendation and obtain a commerce secure web host for $180.00/year. This was passed unanimously.

11. **Public Relations** (Myra Smith)—no report

12. **Archives** (Troy Best)—written letter of request: we still need to obtain photographs (especially members of the Executive Committee), committee reports, minutes of the Executive Committee meetings, etc. Please send these to Dr. Best or Dr. Dwayne Cox, the archivist in charge of AAS materials at the Auburn University Ralph B. Draughton Library.

13. **Science and Public Policy** (Dail Mullins)—written report. The committee membership has now been "stabilized." Dr. Mullins reported that most of the committee's activities this year, as in past years, have focused on the continued monitoring of anti-evolution forces in the state; this year, attention has been drawn to these group's efforts to influence both the composition and deliberations of the recently formed Alabama State Science Textbook Selection Committee. At the request of Ms. Cissy Bennett, a science teacher at Mt. Brook High School, and after consultation with the committee members, the chair of the Science and Public Policy Committee drafted a letter to Governor Don Siegelman on behalf of this committee, endorsing a slate of candidates for the Textbook Committee recommended by the National Association of Biology Teachers.

14. **Gardner Award** (Prakash Sharma)—no report

15. **Carmichael Award** (Velma Richardson)—written report. The committee presented its annual award for the outstanding paper published in the *JAAS* during the previous year to James T. Bradley, H. Shin Shim, and Kelley Moody, Department of Biological Sciences, Auburn University ("Effects of Exogenous Juvenile Hormone on Vitellogenesis in the Cricket, *Acheta domesticus* (L.)"

16. **Resolutions** (Priscilla Holland)—no report

17. **Nominating Committee** (Anne Cusic)—as chair of the nominating committee during the upcoming year, I will be soliciting nominations for the offices that become vacant. These include, but are not limited to, Second Vice-President and four Trustees. With the assistance of Dr. Dute and Dr. Watts, I will prepare a slate of officers to be presented at the Spring Executive meeting. If you, or anyone you know, would be interested in being nominated for any open positions, please contact me.

18. **Mason Scholarship** (Michael Moeller)—last year we had seven completed applications for the William H. Mason Scholarship. After reviewing all application materials, the scholarship committee offered a $1000 scholarship to Jeannine A. Ott. Ms. Ott accepted this award. Also included with the report is a copy of an announcement which the committee plans to send soon to deans in Schools of Science and Education in Alabama announcing a $1000 Fellowship in Science Teaching.

INSTRUCTIONS TO AUTHORS

Editorial Policy: Publication of the *Journal of the Alabama Academy of Science* is restricted to members. Membership application forms can be obtained from Dail W. Mullins, Jr., Honors Program, HOH 105, University of Alabama at Birmingham, 1530 3rd Avenue South, Birmingham, AL 35294-4450. Subject matter should address original research in one of the discipline sections of the Academy: Biological Sciences; Chemistry; Geology: Forestry, Geography, Conservation, and Planning; Physics and Mathematics; Industry and Economics, Science Education; Social Sciences; Health Sciences; Engineering and Computer Science; and Anthropology. Timely review articles of exceptional quality and general readership interest will also be considered. Invited articles dealing with Science Activities in Alabama are occasionally published. Book reviews of Alabama authors are also solicited. Submission of an article for publication in the implies that it has not been published previously and that it not currently being considered for publication elsewhere. Each manuscript will receive at least two simultaneous peer reviews.

Submission: Submit an original and two copies to the editor. Papers which are unreasonably long and verbose, such as uncut theses, will be returned. The title page should contain the author's name, affiliation, and address, including zip code. The editor may request that manuscripts be submitted on a diskette upon their revision or acceptance.

Manuscripts: Consult recent issues of the *Journal* for format. Double-space manuscripts throughout, allowing 1-inch margins. Number all pages. An abstract not exceeding 200 words will be published if the author so desires. Use heading and subdivisions where necessary for clarity. Common headings are: **Introduction** (including literature review), **Procedures** (or **Materials and Methods**), **Results, Discussion,** and **Literature Cited.** Other formats may be more appropriate for certain subject matter areas. Headings should be in all caps and centered on the typed page; sub-headings should be italicized (underlined) and placed at the margin. Avoid excessive use of footnotes. No not use the number 1 for footnotes; begin with 2. Skip additional footnote numbers if one or more authors must have their present address footnoted.

Illustrations: Submit original inked drawings (graphs and diagrams) or clear black and white glossy photographs. Width must not exceed 15 cm and height must not exceed 20 cm. Illustrations not conforming to these dimensions will be returned to the author. Use lettering that will still be legible after a 30% reduction. Designate all illustrations as figures, number consecutively, and cite all figures in the text. Type figure captions on a separate sheet of paper. Send two extra sets of illustrations; xeroxed photographs are satisfactory for review purposes.

Tables: Place each table on a separate sheet. Place a table title directly above each table. Number tables consecutively. Use symbols or letters, not numerals, for table footnotes. Cite all tables in the text.

Literature Cited: Only references cited in the text should be listed under **Literature Cited.** Do not group references according to source (books, periodicals, newspapers, etc.). List in alphabetical order of senior author names. Cite references in the text parenthetically by author-date.

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